Bhumi Nath Tripathi
Dhananjay Kumar
Editors

Prospects and Challenges in Algal Biotechnology

Springer

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ISBN 978-981-10-1949-4 ISBN 978-981-10-1950-0 (eBook)
https://doi.org/10.1007/978-981-10-1950-0

Library of Congress Control Number: 2017954284

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Dedicated to our teacher
Professor J.P. Gaur

Preface

The last two decades have witnessed a great surge in the research on algal biotechnology. Significant advances have been made in areas relating to the use of microalgae in biofuel production, carbon dioxide sequestration, and environmental bioremediation. The culture of microalgae in photobioreactors, harvesting of algal biomass, mixotrophic growth including carbon recovery, and environmental remediation using microalgae, phototrophic biofilms, and mats are the major areas in which a good deal of information has been generated. The enormous potential of algae as the source of food, nutraceuticals, and pharmaceuticals has also been recognized. The present volume endeavors to present a critical account of some of the above-mentioned aspects of algal biotechnology.

The book is organized into 12 chapters. The first two chapters comprise the modeling and event-based control systems for the culturing of microalgae in industrial photobioreactors, while Chapters "Generation and Harvesting of Microalgae Biomass for Biofuel Production" and "Microalgae-Based Biorefineries as a Promising Approach to Biofuel Production" deal with the algal biomass generation, harvesting, and integrated use of microalgae in biorefinery and generation of biofuel feedstock. Chapter "Microalgae Mixotrophic Growth: Opportunity for Stream Depuration and Carbon Recovery" explores the possibility of employing microalgae, growing under the mixotrophic condition, in depuration of stream and recovery of carbon. The next four chapters (Chapters "Sustainable Utilization of Marine Algae Biomass for Environmental Bioremediation"–"Wastewater Treatment Using Phototrophic-Heterotrophic Biofilms and Microbial Mats") are dedicated to discussing the use of microalgae, cyanobacteria, and phototrophic biofilms/mats in environmental bioremediation. The selective metal ion homeostasis in cyanobacteria has been elegantly described in Chapter "Selective Metal Ion Homeostasis in Cyanobacteria". Chapters "Algae as Source of Food and Nutraceuticals"–"Production of Primary and Secondary Metabolites Using Algae" discuss the use of microalgae as the source of food, nutraceuticals, and pharmaceuticals.

We would like to take this opportunity to express gratitude to our teacher Prof. J.P. Gaur, who provided stimulating inspiration, valuable suggestions,

appurtenant criticism, and also showed a keen interest in shaping our career in algal biology. His breadth of vision and deep knowledge of the subject always enlightened our path, so that we could attain academic excellence. We fail to find adequate words to express our humble gratitude to him and dedicate this book to him as a token of our respect for him.

We are thankful to the authors of various chapters of this book for their kind cooperation in completing the task timely. We also appreciate the patience of each one of them for politely complying with various suggestions that we placed before them as the editors of this book. Dr. Madhurima Kahali and Ms. Sowndarya Kumaravel, Editorial Office, Springer, extended valuable help during the preparation and editing of the book. Our colleagues at Indira Gandhi National Tribal University, Amarkantak and H.N.B. Garhwal University, Srinagar–Garhwal, appropriately helped us in various ways. Our research scholars competently assisted us in the reading of the proofs of the various chapters.

BNT is thankful to UGC, CSIR, DST, and DBT for giving financial support through various research projects. DK acknowledges the University Grants Commission, New Delhi for providing financial support in the form of a start-up project.

At last, but not the least, we extend warm appreciation to our wives—Pratima and Gunjan—for their encouragement and patience during the course of the editing of this book.

Amarkantak, India — Bhumi Nath Tripathi
Srinagar-Garhwal, India — Dhananjay Kumar

Contents

Event-Based Control Systems for Microalgae Culture in Industrial Reactors 1
A. Pawlowski, J.L. Guzmán, M. Berenguel, F.G. Acién and S. Dormido

Dynamic Modeling of Microalgal Production in Photobioreactors 49
I. Fernández, J.L. Guzmán, M. Berenguel and F.G. Acién

Generation and Harvesting of Microalgae Biomass for Biofuel Production 89
Md. Asraful Alam, Zhongming Wang and Zhenhong Yuan

Microalgae-Based Biorefineries as a Promising Approach to Biofuel Production 113
Jorge Alberto Vieira Costa, Luiza Moraes, Juliana Botelho Moreira, Gabriel Martins da Rosa, Adriano Seizi Arruda Henrard and Michele Greque de Morais

Microalgae Mixotrophic Growth: Opportunity for Stream Depuration and Carbon Recovery 141
Giuliana D'Imporzano, Salati Silvia, Veronesi Davide, Scaglia Barbara and Adani Fabrizio

Sustainable Utilization of Marine Algae Biomass for Environmental Bioremediation 179
Laura Bulgariu and Dumitru Bulgariu

Selective Metal Ion Homeostasis in Cyanobacteria 219
Lee Hudek and M. Leigh Ackland

Bioadsorption of Heavy Metals 233
Aridane G. González, Oleg S. Pokrovsky, J. Magdalena Santana-Casiano and Melchor González-Dávila

Wastewater Treatment Using Phototrophic–Heterotrophic Biofilms and Microbial Mats 257
J. Paniagua-Michel

Algae as Source of Food and Nutraceuticals 277
Katarzyna Godlewska, Agnieszka Dmytryk, Łukasz Tuhy and Katarzyna Chojnacka

Algae as Source of Pharmaceuticals 295
Agnieszka Dmytryk, Łukasz Tuhy and Katarzyna Chojnacka

Production of Primary and Secondary Metabolites Using Algae 311
Milagros Rico, Aridane G. González, Magdalena Santana-Casiano, Melchor González-Dávila, Norma Pérez-Almeida and Miguel Suarez de Tangil

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Event-Based Control Systems for Microalgae Culture in Industrial Reactors

A. Pawlowski, J.L. Guzmán, M. Berenguel, F.G. Acién and S. Dormido

Abstract In this chapter, event-based control approaches for microalgae culture in industrial reactors are evaluated. Those control systems are applied to regulate the microalgae culture growth conditions such as pH and dissolved oxygen concentration. The analyzed event-based control systems deal with sensor and actuator deadbands approaches in order to provide the desired properties of the controller. Additionally, a selective event-based scheme is evaluated for simultaneous control of pH and dissolved oxygen. In such configurations, the event-based approach provides the possibility to adapt the control system actions to the dynamic state of the controlled bioprocess. In such a way, the event-based control algorithm allows to establish a tradeoff between control performance and number of process update actions. This fact can be directly related with reduction of CO_2 injection times, what is also reflected in CO_2 losses. The application of selective event-based scheme allows the improved biomass productivity, since the controlled variables are kept within the limits for an optimal photosynthesis rate. Moreover, such a control scheme allows effective CO_2 utilization and aeration system energy minimization. The analyzed control system configurations are evaluated for both tubular and raceway photobioreactors to proove its viability for different reactor configurations as well as control system objectives. Additionally, control performance indexes have been used to show the efficiency of the event-based control approaches. The obtained results demon-

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B.N. Tripathi and D. Kumar (eds.), *Prospects and Challenges in Algal Biotechnology*, DOI 10.1007/978-981-10-1950-0_1

strated that the analyzed control algorithms improve the microorganism growth condition and in consequence the overall production rate is increased.

1 Introduction

Microalgae have been proposed for the production of industrial commodities as biofertilizers and biofuels, in addition to CO_2 abatement and wastewater treatment processes (Acién et al. 2012; Kokossis and Yang 2010; Costache et al. 2013; Mendoza et al. 2013b). They can contain more than 50% crude protein, with a 25-fold higher yield than soybeans, and their lipids productivity is several times larger than vegetable oils. Another important factor that characterizes the microalgae process is the high cultivation rate for the surface area used in its growth (Peng et al. 2013). Moreover, microalgae have been proposed as the only alternative for the sustainable production of biodiesel and bioethanol (Chisti 2007). Nonetheless, to be competitive in the commodities, market microalgal production must approximate crop prices and become lower than 0.5 €/kg, much lower than actual production cost (Acién et al. 2012). Thus, in practice, microalgae are used today in animal feed, in addition to human nutrition, cosmetics, and in the production of high-value ingredients such as polyunsaturated fatty acids and carotenoids (Spolaore et al. 2006). The worldwide production of microalgal biomass is 9000 t dry matter per year, its price ranging from 30 to 300 €/kg, and the size of these markets is growing considerably (Brennan and Owende 2010; Charpentier 2009).

The microalgae culture can be cultivated in reactors with different architectures, providing the properties one is looking for. Closed photobioreactors are generally used when high-quality algal biomass from determined strain is required. To this end, tubular photobioreactors are frequently used. When the high production volume is prioritized over the quality, open reactors are employed. In this group, the raceway photobioreactor is the most popular, because of its low production costs and operation simplicity (Weissman and Goebel 1987). Nowadays, there are many architectures that slightly vary from original design proposed by Oswald in the 1960s (Oswald and Golueke 1960). Despite differences in the physical architecture, all of them are developed to provide optimal microalgae growth conditions. As shown in Costache et al. (2013), the most important parameters affecting microalgae culture are pH, solar irradiance, dissolved oxygen, and medium temperature. Additionally, the photosynthesis rate in microalgae growth process depends not only on solar irradiance, but also influenced by other variables resulting in a complex system.

In open configurations, such as raceway reactors, operating temperature as well as light regimen is specified by the photobioreactor architecture and cannot be changed during operation. All remaining parameters of microalgae growth process, e.g., pH and dissolved oxygen, must be managed by suitable control scheme. The dissolved oxygen and pH levels depend on the photosynthesis rate and should be maintained close to their optimal levels. Otherwise, microalgae biomass productivity will drop and in extreme cases could lead to harmful effect on microorganism conditions.

All aforementioned properties indicate the raceway reactor as a feasible solution for microalgal biomass production. Many studies were performed on the optimal raceway reactors design and architectures providing optimal conditions for the microorganism growth (Weissman et al. 1988; Richmond 2004; Chiaramonti et al. 2013; Mendoza et al. 2013b). In consequence, there exist many raceway photobioreactors designs that slightly differ from the design proposed by Oswald and Golueke (1960), providing optimal culture conditions (Chiaramonti et al. 2013; Sompech et al. 2012; Mendoza et al. 2013a, b). In spite of several layouts, the operation principle is similar and numerous factors need to be fulfilled to provide optimal biomass production rate.

Although traditionally microalgae have been cultivated in open photobioreactors due to the simplicity and low cost, when high-value algal products from defined strains are required, it is necessary to use closed photobioreactors such as tubular photobioreactors (Taras and Woinaroschy 2012). These photobioreactors allow to control the operating conditions and avoid contamination, being available to fulfill requirements for the production of biomass in food, feed, and additives (Wang et al. 2012). The main objective of the closed photobioreactor system is to obtain a high-quality biomass by adjusting the culture conditions to optimal values requested by microalgae strain used, especially pH. In tubular photobioreactors, pH control is performed by injection of pure carbon dioxide. The supply of pure carbon dioxide can constitute up to 30% of the overall microalgae production cost (Acién et al. 2012). Moreover, the carbon losses in tubular photobioreactors can be higher than 50% in extreme cases, but can be reduced below 30% through proper design and operation of the photobioreactor (Acién et al. 1999). To reduce this even further, it is necessary to design advanced control strategies that take into account the mixing and mass transfer phenomenon that occurs in the system (García et al. 2003; Sierra et al. 2008; Cai et al. 2013). Thus, the main control problem for these processes will be to regulate the system pH by simultaneously minimizing the CO_2 losses.

Next to temperature and solar irradiance, pH is one of the most important parameters that affect the photosynthesis performance. The application of CO_2 to the microalgae growth process changes the pH level due to acidity alteration of the microorganism growth medium. Moreover, the CO_2 is used also to provide the inorganic carbon to prevent carbon limitation of the photosynthesis production rate. The excess of the CO_2 significantly decreases pH, resulting in culture damage. On the contrary, CO_2 scarcity could reduce drastically the inorganic carbon availability below the required level and result in limited growth performance (Beneman et al. 1987; Berenguel et al. 2004). The use of CO_2 corresponds to the important operational cost for microalgae production process, so it could not be supplied in excess (Godos et al. 2014). Additionally, unlimited CO_2 supply lead to unnecessary emission to the atmosphere and should be optimized by control system with efficient use of resources (Beneman et al. 1987; Pawlowski et al. 2014; Bernard 2011). Using this relationship, the control technique uses the pH level to compute the time instant and the quantity of CO_2 to be supplied. For this reason, the tradeoff between minimization of carbon dioxide losses and pH regulation precision should be taken into account in the control approach. From the economic point of view, the profitabil-

ity of microalgae culture can be improved, if carbon dioxide requirement is covered using waste flue gases (Laws and Berning 1991; Putt et al. 2011; Godos et al. 2014). In this particular configuration, it is necessary to guaranty high mass transfer rates, what has been extensively studied in previous research (Putt et al. 2011; Tang et al. 2011; Godos et al. 2014). The flue gases application method can be performed using two approaches, controlled on-demand supply and continuous bubbling, respectively (Acién et al. 2012). According to the finding from Doucha et al. (2005) and Godos et al. (2014), controlled on-demand supply of waste flue gases provides improved efficiencies of the order of 33% and 66%, respectively. Otherwise, continuous bubbling technique is characterized by low efficacy of carbon dioxide usage, obtaining 4.2 and 8.1% following analysis reported in Hu et al. (1998) and Zhang et al. (2001).

All previous control techniques for pH control provided promising performance results with an adequate CO_2 losses reduction. In Fernández et al. (2010), classical PID and feedforward control strategies were developed to cope with this problem, where a simplified linear model of the pH evolution based on changes in CO_2 injection was used for control design purposes. Moreover, in Romero-García et al. (2012), an FSP (Filtered Smith Predictor) approach has been used to improve control results in the presence of significant time delay due to pH sensor location. In both cases, important improvements against on/off controllers were obtained. On the other hand, MPC (Model Predictive Control) techniques have also been used satisfactorily in pH control problems obtaining very good results (Senthil and Zainal 2012; Oblak and Skrjanc 2010; Lazar et al. 2007). An example of pH control in photobiosreactors using Generalized Predictive Controller (GPC) can be found in Berenguel et al. (2004). In that case, it was possible to improve the overall control performance respect to the classical on/off controller. This was possible, thanks to the predictive algorithm that captures the process dynamics as well as considers the on/off valve limitation explicitly in the process constraints. Besides, CO_2 losses were considerably reduced in comparison to the commonly used on/off controllers. The main advantage of MPC algorithms is their constraints handling capability, allowing to consider any physical limitations of the process as well as the used equipment. For that reason, these algorithms are very popular in chemical process control applications (Hu and Farra 2011; Christofides et al. 2013).

Another parameter that has an important influence on the photosynthesis rate is the dissolved oxygen content. High concentration indexes of this variable in the culture medium lead to a severe threat to microorganism growth. In combination with other parameters, it could provoke light-energy dissipation through photorespiration, enzyme inhibition of the photosynthetic pathways, and might cause damage to the photosynthetic apparatus, membrane structures, DNA, and other cellular components (Peng et al. 2013; Ugwu et al. 2007; Santabarbara et al. 2002; Marquez et al. 1995). In open pounds, such as raceway reactor, it is supposed that no specific control is needed for dissolved oxygen, since its excess should be transmitted to the atmosphere. Nevertheless, in practice, this assumption is not always correct, since dissolved oxygen concentration can reach as high as 500% air saturation as reported in Mendoza et al. (2013a), Peng et al. (2013). To reduce dissolved oxygen influence on microalgae growth, it is necessary to incorporate stirring mechanism or aeration.

In both techniques, it is necessary to increase the complexity of the reactor adding new equipment, what increases production and maintenance costs. Following the results from Peng et al. (2013) and Mendoza et al. (2013a), the dissolved oxygen evacuation problem is still an important challenge despite the significant technical improvements in this subject. Especially, information on large-scale reactors is insufficient, so additional research is required regarding large-scale photobioreactors with dedicated control systems (Han et al. 2012).

However, as these control algorithms are time-based control approaches, the control action is always calculated and executed on the plant even for small control errors. Thus, the control effort, and therefore the CO_2 losses, may be reduced even further if control techniques that only act when necessary (for instance, when the control error is relevant) are used. This is the case of event-based control approaches, which are becoming very popular from a practical point of view since they allow finding a tradeoff between performance and control effort in a very straightforward manner (Årzén 1999; Pawlowski et al. 2009). Due to these properties, an event-based control approach can be applied to wide range of process, since it allows to establish a tradeoff between control effort (resource utilization) and control performance.

The event-based controllers have been introduced as an alternative solution for classical control systems (Årzén 1999; Åström 2007). The main feature that differentiates event-based systems from classical approach is realtered to the ability to adapt itself to the dynamics of controlled process. Several previous works report that usage of event-based control techniques provides many advantages, especially for a wide range of bioprocesses (Beschi and Dormido 2012; Sánchez et al. 2011; Pawlowski et al. 2014). The event-based control systems become an interesting alternative for processes where the compromise (tradeoff) between usage of control resources and control system accuracy must be met. This tradeoff can be linked to economic or environmental aspects, such as energy usage minimization, fuel savings, or any other quantity that need to be optimally used (Pawlowski et al. 2012b, 2014). Some examples can be found in a recently performed work (Pawlowski et al. 2014), which study the event-based control approach applied to pH control in a tubular photobioreactor. The introduced event-based approach reduces carbon dioxide losses, and simultaneously keeping pH inside the established limits being optimal for biomass production. From the industrial production point of view, this aspect is critically important since it prevents excessive dose of waste flue gases that could provoke environmental danger. Considering aforementioned advantages of event-based approaches, in this chapter, we provide the practical evaluation of such control systems that are applied to the tubular as well as raceway industrial photobioreactors.

First analyzed strategy addresses the development and implementation of an event-based MPC algorithm to control the pH of an industrial photobioreactor. From the pH control point of view, the event-based MPC could reduce the controller attention (reducing computational costs) while limiting the resource utilization (applying new control values only if it is strictly necessary). Hence, the application of event-based control in tubular photobioreactors is motivated by reaching both, control effort reduction (minimization of CO_2 losses) and keep the pH level in a certain range. In this case, a recent event-based GPC control strategy (Pawlowski et al.

2012b) is implemented to control the pH in a tubular photobioreactor and to achieve the system requirements. The event detection is based on the level-crossing sampling technique with additional time limits (Årzén 1999). With this idea, the control actions are only calculated when the process output is outside a certain band around the set point. The control law is computed fast when events are detected and slow when there are no events. On the other hand, the controlled variable, pH, is regulated with established tolerance (determined by sensor virtual deadband), what allows to reduce the control effort (Pawlowski et al. 2015). In this configuration, the control variable (state of on/off valve) is updated only when necessary, what reduces considerably the CO_2 usage. This fact is directly reflected in CO_2 loses, which makes the event-based controller a resource-aware control system. The presented control strategy is tested on an industrial photobioreactor to verify its performance against plant-model mismatch, process disturbances, and measurement noise. Moreover, the obtained results are compared with classical on/off controllers and the classical time-based GPC.

The second analyzed approach shows the event-based control approach applied to raceway reactors for pH process control. In such application, the event-based control technique provides improved pH control accuracy, improving the growth condition of microorganisms. Additionally, the event-based GPC in combination with actuator deadband (Pawlowski et al. 2014) minimizes the injection time of flue gases. The developed event-based controller has an additional design parameter to adjust virtual actuator deadband. Additionally, the event-based predictive controller can be extended with disturbance compensation mechanism (Camacho and Bordóns 2007; Pawlowski et al. 2012a). Such a feature has significant importance in the raceway photobioreactor production process, since pH value is continuously influenced by changes in solar irradiance. Moreover, the disturbance handling mechanism implementation allows to improve the pH control accuracy as well as reduce even further the usage of flue gases. Taking into account this relationship, the value of virtual deadband is selected to meet the control performance and provide optimal conditions for microalgae growth. Simultaneously, the tested controller reduces the volume of injected flue gases as much as possible for desired accuracy. In this way, the evaluation of the control scheme is focused on the effective use of waste flue gases. The tests carried out in this study were performed on the industrial-scale raceway photobioreactor with no additional equipment (with basic setup). Anyhow, the proposed event-based controller can be coupled with other devices/techniques (e.g., such as presented in Su et al. 2008) to increase CO_2 transfer rate. Moreover, analyzed event-based GPC control system with actuator virtual deadband is compared to the commonly used control technique used for pH regulation (on/off controller, in this case) in raceway reactor. The comparison between two control techniques is performed through several performance indexes.

Finally, the event-based controller was implemented using selective control approach that can manage simultaneously pH and dissolved oxygen control (Pawlowski and Mendoza 2015; Pawlowski et al. 2016). As mentioned before, there are many aspects that have to be considered in the control system design. From the raceway reactor point of view, the most important are related to aeration

system energy minimization and effective carbon dioxide utilization (Johnsson et al. 2015). It should be highlighted that the control objectives for dissolved oxygen and pH are adversative, since CO_2 assimilation can be deteriorated by the aeration system. Due to these properties, a selective control technique is suitable for this issue, since it allows to limit undesired interactions and merge the objectives. The selective controller permits one to switch between two control approaches subject to logical criteria (Smith 2002; Liptak 2004). Moreover, the provided controller structure should manage the actuation dynamics to process state. This requirement can be satisfied using an event-based system as shown in Beschi et al. (2014), Pawlowski et al. (2012b), Beschi and Dormido (2012), and Pawlowski et al. (2014). In the presented control scheme, we consider all these properties and developed event-based controller is able to handle pH and dissolved oxygen with mentioned constraints.

2 Photobioreactors

This section describes the main information and configuration of the photobioreactors used for the experimental tests in this chapter. Furthermore, the linear models used for control designed purposes are presented.

2.1 Tubular Reactor

The pilot-scale reactor analyzed in this study is located at experimental station "Las Palmerillas" (Almería, Spain—property of CAJAMAR Foundation). The used facility is composed of ten tubular fence-type photobioreactors (detailed description can be found in Acién et al. 2001). The reactor consists of an external vertical loop with airlift pump that moves the culture medium through the vertical solar receiver made of transparent tubes and bubbling column (Fig. 1). The solar irradiance receiver loop has 0.09 m diameter and obtains a total length (horizontal) of 400 m. The reactor total volume is 2600 l and it has 0.7 m width and 19 m length. The receiver loop is designed to reduce the demand for land (occupying min. area), improve the flow, and maximize the penetration of the solar radiation. For the heat exchange and for degassing, the bubble column is used (3.5 m high and 0.4 m diameter). Entering gas and liquid flows are measured with digital meters. Additionally, dissolved oxygen, pH, and the temperature are monitored at the beginning and at the end of the receiver. The constant air flow rate of 20 l/min is applied in the column. At the entrance of solar receiver on-demand injections of pure CO_2 (5 l/min range) are performed to control the culture pH. Moreover, culture medium is recirculated at 0.9 m/s and the temperature is controlled through heat exchanger placed in the bubbling column. The strain used, *Scenedesmus almeriensis* CCAP 276/24 (freshwater), obtains high growth rate, supporting up to $pH = 10$ and temperature up to 45 °C, being temperature 35 °C and $pH = 8$ optimal for its cultivation. The microal-

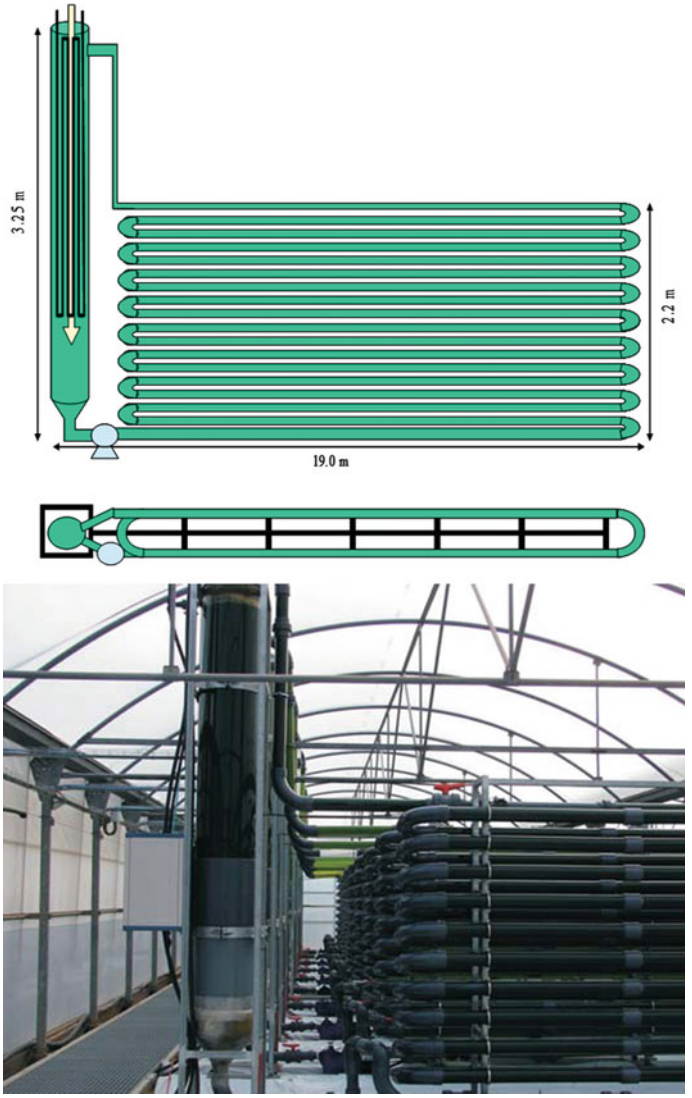


Fig. 1 Tubular photobioreactor: plane and real view (Pawlowski et al. 2014)

gae culture was cultivated in continuous mode (0.34 1/day dilution rate) and medium, Mann&Myers, and was prepared using agricultural fertilizers.

2.1.1 Linear pH Model

In order to design control system for pH variable (that rely on model predictive control approach), a simplified linear model is used. In such a process, the medium pH is influenced by CO_2 uptake and CO_2 supply that are affected by photosynthesis rate. The supplied CO_2 is used to decrease the pH level due to the formation of carbonic acid. On the contrary, the pH value gradually rises, since during photosynthesis process microalgae consume CO_2 and produce O_2 affecting medium acidity. Moreover, the photosynthesis rate depends on the solar radiation also resulting in pH changes. In this process, the pH value is the controlled variable and CO_2 supply flow is the control variable. Additionally, in such a configuration, the solar radiation is main control system disturbance. This scheme can be captured using linear model representation (Berenguel et al. 2004; Sierra et al. 2008; Fernández et al. 2010).

The parameters of such model were identified taking into account the dynamics observed in measurements and also considering distributed nature of photobioreactor architecture. The resulting model represents interaction between CO_2 injections and the pH value. Considering that microalgae culture process is nonlinear, the linear models were identified around operation point. The obtained transfer functions are as follows (Berenguel et al. 2004; Fernández et al. 2010):

$$pH = \underbrace{\frac{k_1}{1+\tau_1 s}}_{TF_1(s)} \underbrace{\frac{\omega_n^2}{s^2+2\delta\omega_n s+\omega_n^2} e^{-t_r s}}_{TF_2(s)} u + \underbrace{\frac{k_r}{1+\tau_r s}}_{TF_3(s)} I. \tag{1}$$

In this case, pH is the microalgae culture pH, u is the control variable (CO_2 supplying flow value, 0–100%), and I is the solar radiation (measurable control system disturbance). In this configuration, TF_1 is a first-order term relating input and output variable, TF_2 is used to capture recirculation effect, and t_r is a time delay present in the system due to distance between CO_2 injection point and the pH sensor. Moreover, TF_3 describes disturbance effect on the controlled variable. The final model parameters are as follows: $k_1 = -0.08$ pH $\%^{-1}$, $\tau_1 = 28$ min, $\omega_n = 0.014$ rad s^{-1}, $\delta = 0.042$, $t_r = 7$ min, $k_r = 0.002$ pH m^2 W^{-1}, and $\tau_r = 182$ min. The dynamic response of the obtained model can be seen in Fig. 2, which is compared with the response of real system.

2.2 *Raceways Reactor*

The study for raceway reactor was performed on the pilot-scale facilities located at experimental station "Las Palmerillas" (Almería, Spain—property of CAJAMAR Foundation). Used reactor has 100 m^2 surface area and is composed of two 50-m-long (1 m wide) channels forming U-shape bends (see Fig. 3). The reactor operates at constant depth (0.2 m) to provide desired performance and considering power

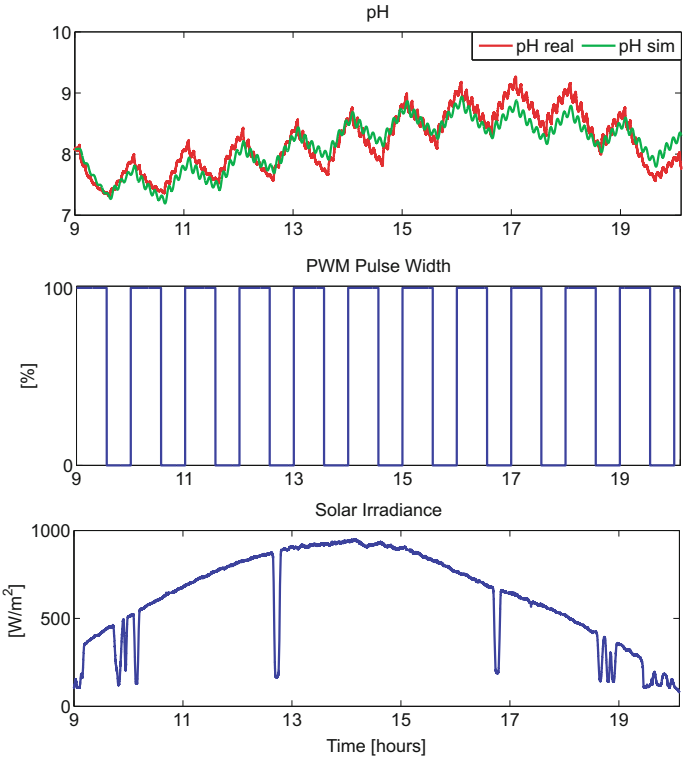
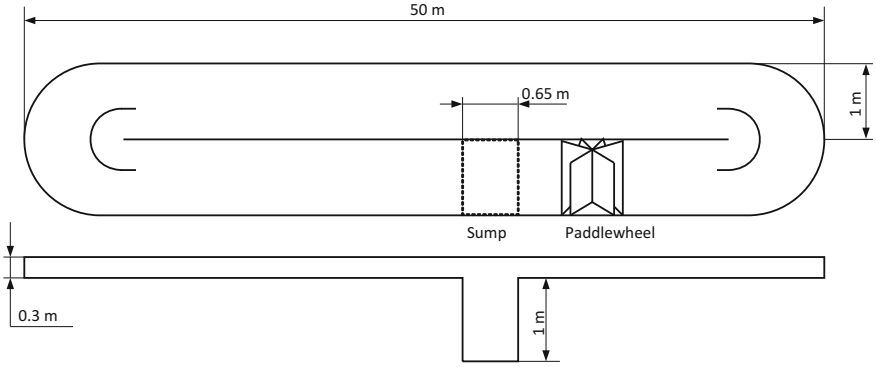


Fig. 2 Simplified model validation results for the tubular photobioreactor (Pawlowski et al. 2014)

consumption issues. Resulting reactor volume is 20 m^3. Medium mixing was performed using paddlewheel (1.2 m diameter) with eight marine plywood blades at constant speed. Carbonation was done through, 1 m by 0.64 m, sump situated 1.8 m downstream the paddlewheel location. Three plate membrane diffusors were placed at the bottom of the sump and were used to inject flue gases.

The raceway photobioreactor facilities can be set up to use different carbon dioxide sources in order to provide flexible platform to evaluate different systems and techniques. Nevertheless, for the test performed in this analysis, the plant was configured to use flue gases resulting in the raceway reactor operation scheme shown in Fig. 3.

The flue gas used for pH control was taken from industrial heating boiler (diesel-fuelled with the average gas composition: 10.6% CO_2, 18.1 ppm CO, 38.3 ppm NOx, and 0.0 ppm SO_2). In this operation scheme, exhaust gas was refrigerated to the environment temperature and stored in a 1.5-m^3 pressure tank (compressed to 2 bar) with automatic regulation of pressure. The compressed flue gas was supplied to the reactor through a 150 m pipeline of 40 mm diameter. Finally, the injection system uses the solenoid on/off valve and input flow was measured with digital flow meter



(a) scheme (top and side view)

(b) real experimental facilities

Fig. 3 Raceway reactor (Pawlowski et al. 2014)

(detailed information for raceway setup can be found in Godos et al. 2014; Mendoza et al. 2013b). The injection instant as well as aperture time is provided by the control algorithm used for pH control.

Taking into account the limitation of the actuation system that is used in the pilot-scale raceway reactor, it is necessary to convert the continuous control signal into the on/off actions of solenoid control valve. To this end, PWM (Pulse Width Modulation) technique is used to translate the signal provided by the controller into train of pulses with modulated width. The value of the pulse width is determined by the control system and can vary between 0 and 100%. Moreover, the modulation frequency was set to 0.1 Hz being optimal for the main controlled variable. The conversion is performed using software procedure developed as part of a SCADA (Supervisory, Control And Data Acquisition) program.

The dissolved oxygen concentration is controlled using compressed air to evacuate its excess. The compressed air is stored in the high-pressure tank and is supplied to the raceway reactor through the sparger using on/off valve (using the same structure as for CO_2 injections). The dissolved oxygen concentration is measured using 5083T, Crison probes, MM44, and Crison transmitters.

All control techniques tested on the raceway reactor were developed in Matlab environment and implemented as a part of SCADA system. The input and output signals were governed using LabJack U12 modules from LabJack Corp.

2.2.1 Linear Process Model for pH

In the case of raceway reactor, pH process also was modeled using linear reduced-order model. As in the previous reactor type, the model structure is developed considering distribution of the actuators and sensor, the reactor architecture, and dominant dynamics in measured data. The developed model relates the carbon dioxide injections (input variable) with pH (output variable) and is given by the following structure (s represents the complex variable used in Laplace transform):

$$pH(s) = \underbrace{\frac{k_1}{1+\tau_1 s} e^{-t_r s}}_{TF_1(s)} u(s) + \underbrace{\frac{k_r}{1+\tau_r s}}_{TF_2(s)} v(s) \tag{2}$$

where pH is the culture medium pH, u is the control variable, and v is the solar radiation. The first term, TF_1, relates the pH level to CO_2 injections. t_r refers to the time delay between the CO_2 injection point and the pH measure point. The TF_2 term captures the solar radiation effect on pH value, and this relationship is expressed as the first-order system. Through process identification and validation procedures, the following parameters were obtained: $k_1 = -0.005$ pH%$^{-1}$, $\tau_1 = 16.5$ min, $t_r = 7$ min, $k_r = 0.0007$ pH m^2 W^{-1}, and $\tau_r = 118$ min. The obtained model fit can be seen in Fig. 4, where the real system data are contrasted with those obtained with developed model (Berenguel et al. 2004; Pawlowski et al. 2014).

It needs to be highlighted that during model validation stage the dissolved oxygen variable was uncontrolled (see the third plot in Fig. 4). From this plot, it can be observed that its measured value reach 400 [%Sat], being significantly over the value that guarantees efficient photosynthesis process. This simple example shows the necessity to develop a control system to deal with this issue. Notice that the pH and dissolved oxygen values are decoupled from the control system point of view, since there is no mutual interaction.

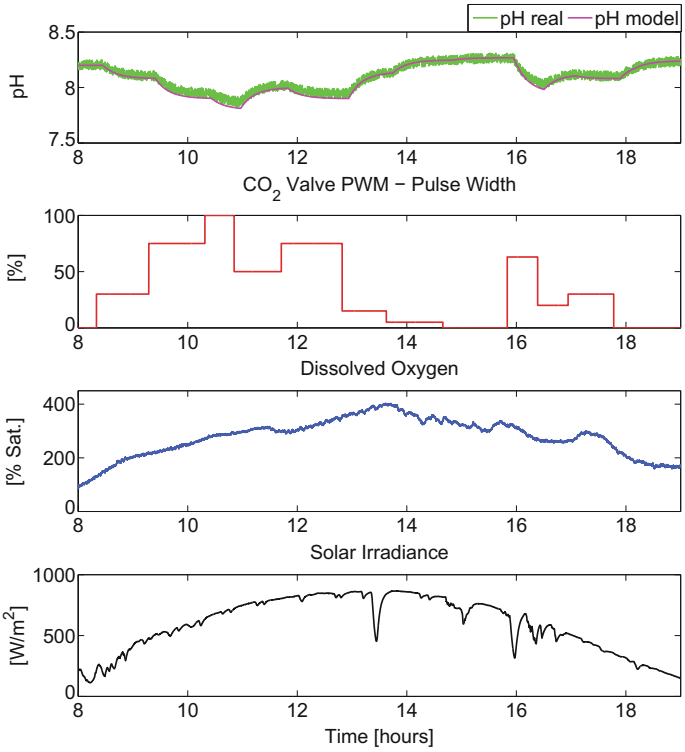


Fig. 4 Simplified model validation results for the raceway photobioreactor (Pawlowski and Mendoza 2015)

3 Control System Objectives

The analysis presented in this chapter focuses on the dissolved oxygen and pH parameters and it is supposed that remaining variables are satisfied through proper photobioreactor design. In this section, detailed information regarding dissolved oxygen and pH effect on the photosynthesis performance is provided. In the previous work (Costache et al. 2013), it was shown that the pH in the 7.0–9.0 range is optimal for the photosynthesis performance. From Fig. 5, it can be observed that in this pH range only insignificant variations in photosynthesis rate were measured. This relationship can be modeled using an Arrhenius expression of the following form:

$$RO2(pH) = B_1 e^{\left(\frac{-C_1}{pH}\right)} B_2 e^{\left(\frac{-C_2}{pH}\right)},$$

where the photosynthesis rate ($RO2$) and $B_1 = 2.50$, $B_2 = 533$, $C_1 = 6.45$, and $C_2 = 69.2$ were obtained by fitting experimental data (Costache et al. 2013).

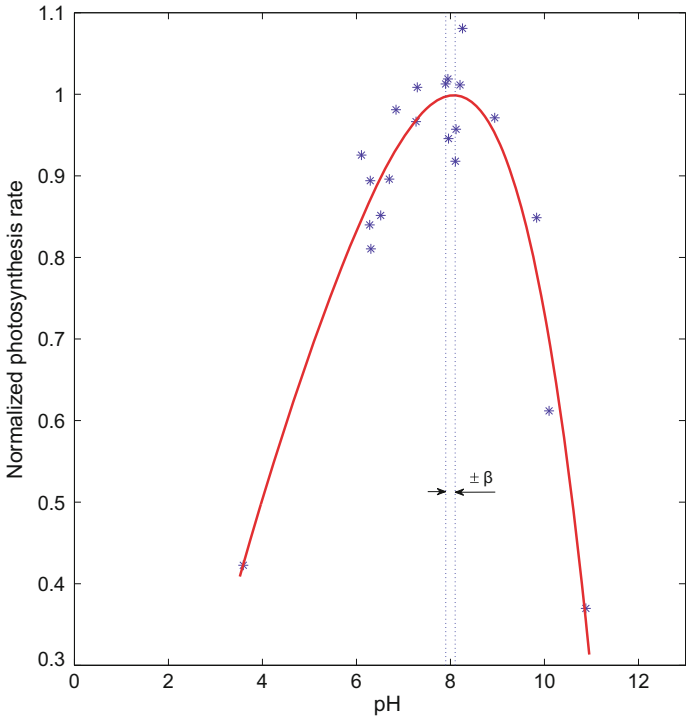


Fig. 5 The photosynthesis rate versus pH of *S. almeriensis* culture at 200 μE/m^{2}s 25 °C, and 100 %Sat of dissolved oxygen (Pawlowski and Mendoza 2015)

Taking into account these features in the control system design, it is possible to regulate the pH around 8 using carbon dioxide injections as a manipulated variable. In Pawlowski et al. (2014), it was demonstrated that slight deviation in reference tracking could provide some benefit for control system goals. The small tolerance (marked as β in Fig. 5) in pH control accuracy does not affect the photosynthesis performance. Due to this property and exploiting event-based control system, it was possible to determine a tradeoff between the injected volume of CO_2 and control performance. Additionally, it should be highlighted that the control of pH level in a raceway reactor is performed using waste flue gases as the carbon dioxide source. For this reason, its usage should be optimized restricting its overdosage.

The relationship between the photosynthesis rate and dissolved oxygen concentration is presented in Fig. 6. As demonstrated in Costache et al. 2013, at dissolved oxygen concentrations lower or equal to saturation (9.0 [mg/l], 100 [%Sat]), the photosynthesis rate is optimal. Nevertheless, dissolved oxygen concentration at higher level reduces exponentially photosynthesis rate reaching zero above (32 [mg/l], 355 [%Sat]), see Fig. 6 for details. The dependence between dissolved oxygen (DO) and the photosynthesis rate can be expressed as follows:

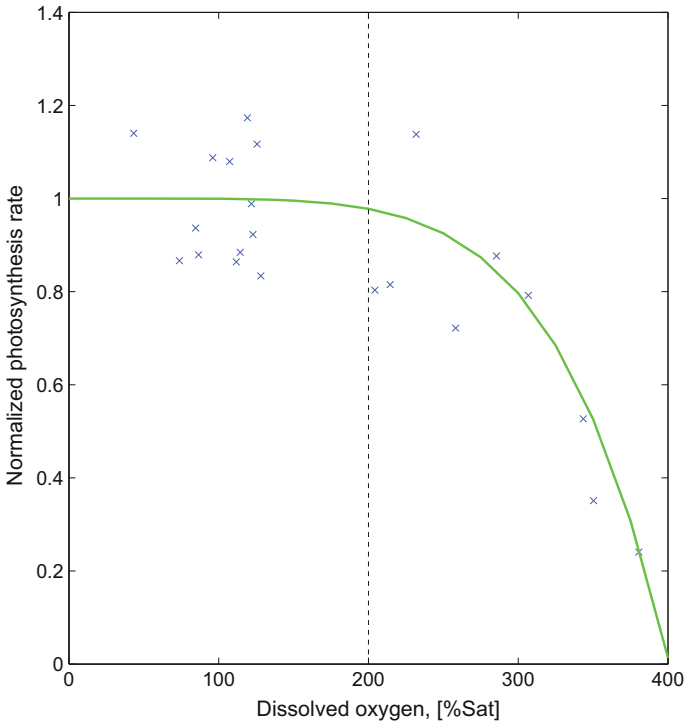


Fig. 6 The photosynthesis rate versus dissolved oxygen concentration of *S. almeriensis* culture at 200 μE/m^{2}s 25 °C, and pH = 8 (Pawlowski and Mendoza 2015)

$$RO2(DO_2) = 1 - \left(\frac{DO_2}{KO_2}\right)^z,$$

where $KO_2 = 32.8$ [mg/l] is the oxygen inhibition constant, and $z = 5.49$ (as reported in Costache et al. 2013). Moreover, the photosynthesis performance decreases only 20%, when dissolved oxygen concentration is up to values of 23 [mg/l] (250 [%Sat]), and above this level the reduction is significantly greater. For this reason, high dissolved oxygen concentration (above 250 [%Sat]) must be evaded irrespective of reactor configuration (Costache et al. 2013; Posten 2009; Camacho et al. 1999). To avoid this situation, the aeration techniques are commonly used. The most important disadvantage of such solution is related to the energy used to supply and compress the air. This issue is of high importance for raceway photobioreactors maintenance costs, since they must be kept low. Considering these facts, application of on-demand injection scheme through a proper control approach is the feasible solution from an economic point of view.

4 Event-Based Control Systems for Microalgae Culture

The event-based control schemes evaluated in this chapter are based on the GPC algorithm with sensor and actuator deadbands, originally published in Pawlowski et al. (2012b, 2014), respectively. Both approaches have been modified, to cope with microalgae culture in industrial photobioreactor. Additionally, the selective event-based approach introduced in Pawlowski and Mendoza (2015) was adapted to cope with simultaneous control of pH and dissolved oxygen within event-based scheme.

The main difference between the event-based and the time-based controller is the ability to adapt the controllers invocation, based on the controlled variable dynamics. In a control system with sensor deadband, the event-based controller will update the system in a fast way, when the controlled variable is an outside established band. Otherwise, when the controlled variable is inside the band, the event-based controller will switch to the slowest sampling in order to reduce the control effort. Considering this working principle, the event-based control structure can manage the control effort adapting it to the performance requirements of the controlled process. In a case of actuator deadband, the main idea consists of a control structure where the control signal is updated in an asynchronous manner. The main goal is to reduce the number of control signal updates, saving system resources, while retaining acceptable control performance.

4.1 Classic Generalized Predictive Controller

The Generalized Predictive Control (GPC) consists of applying a control sequence that minimizes a multistage cost function of the following form:

$$J = \sum_{j=N_1}^{N_2} [\hat{y}(t+j|t) - w(t+j)]^2 + \sum_{j=1}^{N_u} \lambda(j)[\Delta u(t+j-1)]^2, \tag{3}$$

where $\hat{y}(t+j|t)$ is an optimum j step ahead prediction of the system output on data up to time t, $\Delta u(t+j)$ is the future control signal increments, N_1 and N_2 are the minimum and maximum prediction horizons, N_u is the control horizon, $\lambda(j)$ is the control effort weighting sequence, and $w(t+j)$ is the future reference trajectory (Camacho and Bordóns 2007).

The minimum of J, assuming there are no constraints on the control signals, can be found by making the gradient of J equal to zero. Nevertheless, most physical processes are subjected to constraints and the optimal solution can be obtained by minimizing the quadratic function:

$$J(\mathbf{u}) = (\mathbf{Gu} + \mathbf{Pv} + \mathbf{f} - \mathbf{w})^T(\mathbf{Gu} + \mathbf{Pv} + \mathbf{f} - \mathbf{w}) + \lambda \mathbf{u}^T\mathbf{u}, \tag{4}$$

where $\mathbf{G}$ and $\mathbf{P}$ are the matrices containing coefficients of the input–output and disturbance–output step responses, respectively, $\mathbf{f}$ is the free response of the system, $\mathbf{w}$ is the future reference trajectory, $\mathbf{u}$ is the control signal, and $\mathbf{v}$ is the measurable disturbance (Pawlowski et al. 2012a, 2016). Equation (4) can be written in quadratic function form:

$$J(\mathbf{u}) = \frac{1}{2}\mathbf{u}^T\mathbf{H}\mathbf{u} + \mathbf{b}^T\mathbf{u} + \mathbf{f}_0, \tag{5}$$

where $\mathbf{H} = 2(\mathbf{G}^T\mathbf{G} + \lambda\mathbf{I})$, $\mathbf{b}^T = 2((\mathbf{P}\mathbf{v}+\mathbf{f}-\mathbf{w})^T\mathbf{G})$, and $\mathbf{f}_0 = (\mathbf{P}\mathbf{v}+\mathbf{f}-\mathbf{w})^T(\mathbf{P}\mathbf{v}+\mathbf{f}-\mathbf{w})$. The obtained quadratic function is minimized, subject to system constraints, and a classical Quadratic Programming (QP) problem must be solved. The constraints acting on a process can originate from amplitude limits in the control signal, slew rate limits of the actuator, and limits on the output signals, and can be expressed in the short form as $\mathbf{R}\Delta\mathbf{u} \leqslant \mathbf{r}$ (Camacho and Bordóns 2007).

4.2 Event-Based GPC with Sensor Deadband

This section briefly summarizes the event-based GPC algorithm used in this analysis and that was developed in Pawlowski et al. (2012b). In a general way, an event-based controller consists of two parts: an event detector and a controller (Åström 2007). The event detector deals with informing the controller when a new control signal must be calculated due to the occurrence of a new event. In this scheme, the controller is composed of a set of GPC controllers, in such a way that one of them will be selected according to the time instant when a new event is detected, such as described below. The complete control structure is shown in Fig. 7, including the process, the actuator, the controller, and the event generator. This scheme operates using the following ideas:

- The process output is sampled using a constant sampling time T_{base} at the event generator block, while the control action is computed and applied to the process using a variable sampling time T_f, which is determined by an event occurrence.
- T_f is the multiple of T_{base} ($T_f = fT_{base}, f \in [1, n_{max}]$) and verifies $T_f \leq T_{max}$, being $T_{max} = n_{max}T_{base}$ the maximum sampling time value. This maximum sampling time will be chosen to maintain a minimum performance and stability margins.
- T_{base} and T_{max} are defined considering process data and closed-loop specifications, following classical methods for sampling time choice.
- After applying a control action at time t, the process output is monitored by the event generator block at each base sampling time, T_{base}. This information is used by the event detector block, which verifies if the process output satisfies some specific conditions. If these conditions are satisfied, an event is generated with a sampling period T_f and a new control action is computed. Otherwise, the control action is only computed by a timing event, at $t = t + T_{max}$.
- Notice that according to the previous description, the control actions will be computed based on a variable sampling time, T_f. For that reason, a set of GPC con-

trollers is used, where each GPC controller is designed for a specific sampling time $T_f = fT_{base}, f \in [1, n_{max}]$. On the other hand, resampling of the signals is necessary to avoid undesirable jumps in the control action at each change among controllers.

4.2.1 Event-Based Signal Sampling

From the scheme in Fig. 7, it can be seen that the event-based sampling is governed by the event generator block. This block includes two different kinds of conditions in order to generate new events. When one of those conditions becomes true, a new event is generated, and then the current signal values of the process variables are transmitted to the controller block being used to compute a new control action (CO_2 injections in this case). The first condition focuses on checking the process variables. These conditions are based on the level-crossing sampling technique (Miskowicz 2006; Ferre et al. 2010), that is, a new event is considered when the absolute value between two signals is bigger than a specific limit β. For instance, in the case of the set point tracking, the condition would be the following:

$$|w(t) - y(t)| > \beta \tag{6}$$

trying to detect that the process output, $y(t) = pH(t)$, is tracking the reference, $w(t) = w_{pH}(t)$, within a specific tolerance β. The second condition is a time-based condition, used for stability and performance reasons. This condition defines that the maximum period of time between two control signals computation, and thus between two consecutive events, is given by T_{max}:

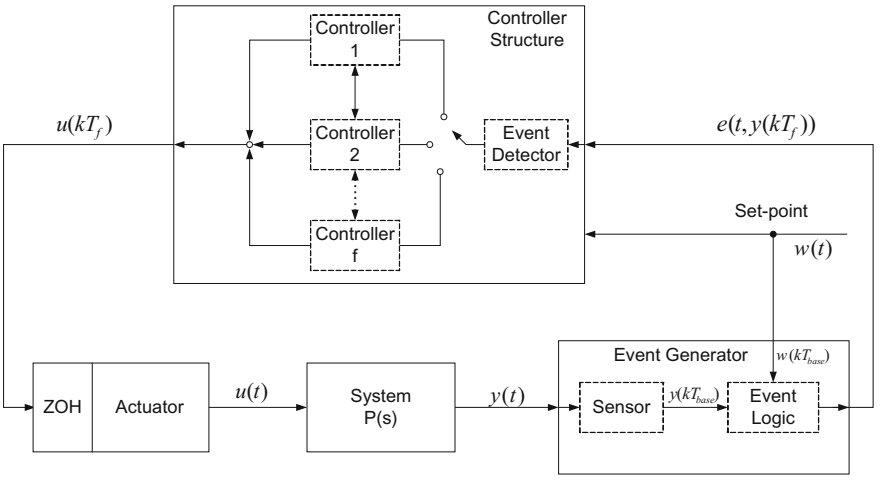


Fig. 7 Event-based GPC control scheme (Pawlowski et al. 2012b)

$$t - t_{e_i} \geq T_{max}, \tag{7}$$

where t_{e_i} is the time when the last event e_i was generated.

These conditions are checked with the smallest sampling rate T_{base}, where the detection of an event will be given within a variable sampling time $T_f = fT_{base}, f \in [1, n_{max}]$. Notice that this variable sampling period determines the current closed-loop sampling time to be used in the computation of the new control action.

4.2.2 Signal Sampling and Resampling Technique

Such as described above, the computation of a new control action is done with a variable sampling period T_f. So, in order to implement the GPC control algorithm, the past values of the process variables and those of the control signals must be available sampled with T_f. Therefore, a resampling of the corresponding signals is required (Pawlowski et al. 2012b, 2014b).

- Resampling of process output: As discussed previously, the controller block only receives the new state of the process output when a new event is generated. This information is stored in the controller block and is resampled to generate a vector y^b including the past values of the process output with T_{base} samples. The resampling of the process output is performed by using a linear approximation between two consecutive events and afterward, this linear approximation is sampled with the T_{base} sampling period, resulting in $y^b(k)$ with $k = 0, T_{base}, 2T_{base}, 3T_{base}, \ldots$. Once the process output signal is resampled, the required past information must be obtained according to the new sampling time T_f, resulting in a new signal, y^f, with the past information of the process output every T_f samples.
  Hence, the vector y^f is obtained as a result, which contains the past process information with the new sampling period T_f to be used in the calculation of the current control action.
- Reconstruction of past control signals: The procedure is similar to that described for the resampling of the process output. There is a control signal, u^b, which is always used to store the control signal values every T_{base} samples. Nevertheless, the procedure for the control signal is done in the opposite way than for the process output. First, the required past information is calculated and afterward, the signal u^b is updated. Let us consider that a new event is generated, what results in a new sampling period $T_f = fT_{base}$. Now, the past information for the new sampling period, T_f, is first calculated from the past values in u^b and stored in a variable called u_p^f. Afterward, this information, together with the past process output data given by y^f, will be used to calculate the new control action, $u^f(T_f) = u^b(k)$. Once the new control action has been computed, $u^f(T_f) = u^b(k)$, the u^b signal is updated by keeping constant the values between the two consecutive events.

4.3 Event-Based GPC with Actuator Deadband

The basic motivation to develop this approach is related to control scheme where the controlled process is updated on an asynchronous basis. The objective is to decrease the amount of control signal changes (reducing resource utilization) simultaneously providing required control accuracy. The actuator virtual deadband is implemented as a constraint on control signal changes: $|\Delta u(t)| = |u(t-1) - u(t)| \geqslant \beta_u$, where β_u is the proposed virtual deadband. In such a scheme, the virtual deadband value is used as an additional degree of freedom in controller tuning procedure. Its value will determine the amount of events related to actuator node, resulting in reduced number of control signal transmissions. In analyzed scheme, the proposed actuator deadband is considered in process model and consequently used in the control signal computation (MPC optimization procedure), which improves control accuracy of event-based approach.

The methodology used in this scheme consists of including the actuator virtual deadband into the GPC design framework (Pawlowski et al. 2014). The deadband nonlinearity can be handled together with other constraints on controlled process. The deadband can be expressed mathematically with a hybrid design framework developed by Bemporad and Morari (1999), which allows to translate discrete rules into a set of linear logical constraints. The resulting formulation consists in a system containing continuous and discrete components, which is known as a Mixed Logical Dynamic (MLD) system (Bemporad and Morari 1999).

Introducing two logical variables, φ_1 and φ_2, to determine a condition on control signal increments, $\Delta u(t)$, these logical variables are used to describe the different stages of the control signal with respect to the deadband, as follows:

$$x(t) = \begin{cases} \Delta u(t) : \Delta u(t) \geqslant \beta_u & \varphi_2 = 1 \\ 0 : \Delta u(t) \leqslant \beta_u & \varphi_2 = 0 \\ 0 : \Delta u(t) \geqslant -\beta_u & \varphi_1 = 0 \\ \Delta u(t) : \Delta u(t) \leqslant -\beta_u & \varphi_1 = 1 \end{cases} \tag{8}$$

To make this solution more general, minimal m and maximal M values for control signal increments are included into the control system design procedure, resulting in $M = max\{\Delta u(t)\}$ and $m = min\{\Delta u(t)\}$. In this way, it is possible to determine the solution region based on binary variables. Thus, the proposed logic determined by Eq. (8) can be translated into a set of *mixed-integer linear inequalities* involving both continuous, $\Delta u \in \mathbb{R}$, and logical variables $\varphi_i \in \{0, 1\}$. Finally, a set of mixed-integer linear inequalities constraints for the actuator deadband is established as follows:

$$\begin{aligned}
\Delta u - (M - \beta_u)\varphi_2 &\leqslant \beta_u \\
\Delta u + (M + \beta_u)\varphi_1 &\leqslant M \\
\Delta u - M\varphi_2 &\leqslant 0 \\
-\Delta u + (m + \beta_u)\varphi_1 &\leqslant \beta_u \\
-\Delta u - (m - \beta_u)\varphi_2 &\leqslant -m \\
-\Delta u + m\varphi_1 &\leqslant 0 \\
\varphi_1 + \varphi_2 &\leqslant 1.
\end{aligned}$$

The reformulated hybrid input constraints presented above are integrated in the GPC optimization problem, where the resulting formulation belongs to an MIQP optimization problem. In the case where the control horizon is $N_u > 1$, the corresponding matrix becomes

$$\underbrace{\begin{bmatrix}
1\underline{\mathbf{D}} & 0\underline{\mathbf{D}} & -(M-\beta_u)\underline{\mathbf{D}} \\
1\underline{\mathbf{D}} & (M+\beta_u)\underline{\mathbf{D}} & 0\underline{\mathbf{D}} \\
1\underline{\mathbf{D}} & 0\underline{\mathbf{D}} & -M\underline{\mathbf{D}} \\
-1\underline{\mathbf{D}} & (m+\beta_u)\underline{\mathbf{D}} & 0\underline{\mathbf{D}} \\
-1\underline{\mathbf{D}} & 0\underline{\mathbf{D}} & -(m-\beta_u)\underline{\mathbf{D}} \\
-1\underline{\mathbf{D}} & m\underline{\mathbf{D}} & 0\underline{\mathbf{D}} \\
0\underline{\mathbf{D}} & 1\underline{\mathbf{D}} & 1\underline{\mathbf{D}}
\end{bmatrix}}_{C}
\underbrace{\begin{bmatrix}
\Delta u\underline{\mathbf{d}} \\
\varphi_1\underline{\mathbf{d}} \\
\varphi_2\underline{\mathbf{d}}
\end{bmatrix}}_{x}
\leqslant
\underbrace{\begin{bmatrix}
\beta_u\underline{\mathbf{d}} \\
M\underline{\mathbf{d}} \\
0\underline{\mathbf{d}} \\
\beta_u\underline{\mathbf{d}} \\
-m\underline{\mathbf{d}} \\
0\underline{\mathbf{d}} \\
1\underline{\mathbf{d}}
\end{bmatrix}}_{\rho},$$

where $\underline{\mathbf{D}}$ is a matrix $(N_u \times N_u)$ of ones and $\underline{\mathbf{d}}$ is a vector of ones with size $(N_u \times 1)$. The previous matrices that contain linear inequality constraints can be expressed in a general form as

$$\mathbf{C}x \leqslant \rho \tag{9}$$

with $\mathrm{x} = [x_c, x_d]^T$, where x_c represents the continuous variables Δu and x_d are those of the logical variables φ_i. Introducing the matrix $\mathbf{Q}_{(3N_u \times 3N_u)}$ and $\mathbf{l}_{(3N_u \times 1)}$ defined as

$$\mathbf{Q} = \begin{bmatrix}
\mathbf{H} & \underline{\mathbf{0}} & \underline{\mathbf{0}} \\
\underline{\mathbf{0}} & \underline{\mathbf{0}} & \underline{\mathbf{0}} \\
\underline{\mathbf{0}} & \underline{\mathbf{0}} & \underline{\mathbf{0}}
\end{bmatrix}; \mathbf{l} = \begin{bmatrix}
\mathbf{b} \\
\hat{0} \\
\hat{0}
\end{bmatrix}, \tag{10}$$

where $\underline{\mathbf{0}} = N_u \times N_u$, $\hat{0} = N_u \times 1$ both of zeros, $\mathbf{H}$ and $\mathbf{b}$ are a matrices used in classical QP optimization, the GPC optimization problem is expressed as

$$\min_x x^T\mathbf{Q}x + \mathbf{l}^T x \tag{11}$$

subject to (9), which is a *Mixed-Integer Quadratic Programming* (MIQP) optimization problem (Bemporad and Morari 1999). The optimization problem involves a quadratic objective function and a set of mixed linear inequalities. Moreover, the classical set of constraints $\mathbf{R}\Delta u \leq \mathbf{r}$ can also be included into the optimization procedure, introducing an auxiliary matrix $\hat{R}$ of the form $[R\ \overline{0}\ \overline{0}]$, where $\overline{0}$ is a matrix of

zeros with the same dimensions that R. Finally, all constraints that must be considered into the optimization procedure are grouped in

$$\begin{bmatrix} C \\ \hat{R} \end{bmatrix} x \leqslant \begin{bmatrix} \rho \\ r \end{bmatrix}.$$

In such a way, the event-based GPC with actuator deadband obtains optimal control signal values considering established deadband and classical constraints.

4.4 Event-Based Selective Control

Considering all aforementioned system aspects, it is necessary to meet all goals through proper control strategy, simultaneously increasing the biomass production performance handling process limitations. Taking into account process features and raceway photobioreactor configuration classical MIMO (Multiple-Input-Multiple-Output), control approach cannot be applied. However, MIMO control approach can be feasible for new reactor design that provides necessary features and solutions. Due to these properties, a selective control is considered for dissolved oxygen and pH control allowing simultaneous control of both variables. Additionally, this control technique is commonly used for controller synchronization in the systems where many control goals exist and all controllers use only one control variable (Liptak 2004). Through a selective control application, it is possible to commute between several controllers in order to optimize controlled process (Smith 2002). Exploiting these features, selective control algorithm can be used to commute between pH and dissolved oxygen controllers simultaneously meeting the control objectives. The developed selective control approach is shown in Fig. 8. From the provided scheme, it can be observed that dissolved oxygen and pH are handled with corresponding controllers, C_{DO} and C_{pH}, respectively. These controllers compute corresponding control action, given as u_{pH} and u_{DO} signals. However, the control signal application

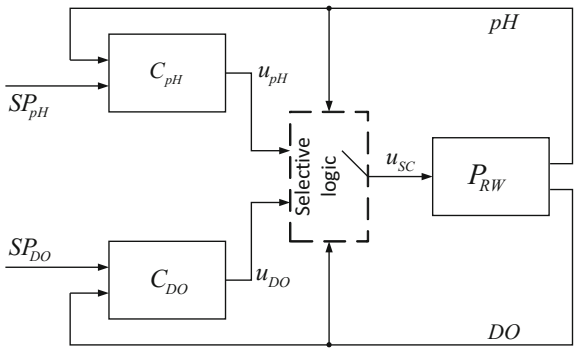


Fig. 8 Selective control scheme for raceway reactor (Pawlowski and Mendoza 2015)

is managed by selective logic (selective mechanism), which determines the signal to be sent to the process (P_{RW}). In the presented approach, the event-based scheme is used to implement selective logic allowing dynamical adaptation to plant state. In the selective mechanism, a deadband sampling technique is used, being very common sampling approach in event-based systems. Finally, the control signal is selected in the following way:

$$u_{SC} = \begin{cases} u_{pH} : |SCSP_{pH} - pH| > \beta \\ u_{DO} : |SCSP_{pH} - pH| < \beta \ \& \ DO > SCSP_{DO} \end{cases} \tag{12}$$

where $SCSP_{DO}$ and $SCSP_{pH}$ are the set points for dissolved oxygen and pH, respectively, β determines the deadband value (control tolerance) for pH control, and DO and pH are the dissolved oxygen and pH and DO values, respectively. Due to these selection criteria, the control signal for pH variable is prioritized always when pH measurement is outside the established band. On the contrary, when pH is inside the band, the selective logic will switch to dissolved oxygen controller.

The selective control structure is developed using previously described scheme. In this configuration, the pH controller uses the event-based approach, which is built on the generalized predictive control using actuator virtual deadband (refer to Sect. 4.3) (Pawlowski et al. 2014). Moreover, the dissolved oxygen control system is based on on/off regulator. This scheme was used due to its simplicity that matches the equipment available for dissolved oxygen control—on/off solenoid valve. In the presented scheme, both pH and dissolved oxygen control systems use the reactor hardware setup with only small modifications in its original structure. The economic cost of performed modifications is negligible regarding the overall economics of the raceway reactor design and operation, since no structural modification was required. For these reasons, both control tasks share the photobioreactor systems in order to perform dissolved oxygen and pH control actions. Considering all these properties, it is possible to separate two reasons why simultaneous control of dissolved oxygen and pH is limited:

- The first reason is related to specific control system goals and limitations in microorganism cultivation system. The photobioreactor pH level is regulated applying CO_2 injections, which affects the acidity of cultivation medium. The carbon dioxide source can vary and depends on the reactor type and final product requirements. Nevertheless, independent on CO_2 source, the volume of injected gas should be optimized to prevent unnecessary emissions and thus environmental contamination. Moreover, dissolved oxygen control focuses on the evacuation of excess oxygen production that appears as a consequence of photosynthesis process. In dissolved oxygen control task, compressed air is injected to the raceway photobioreactor, which results in the reduction of oxygen saturation in cultivation medium. Due to this working principle, the control objectives for pH and dissolved oxygen are antithetical. In the described scenario, the injected air, used to reduce the dissolved oxygen concentration, can also evacuate carbon dioxide not absorbed

by the microorganism. Beside this relationship, both variables present no mutual interference and are decoupled from a control point of view.
- The second refers to the raceway photobioreactor architecture and it is related to application method of the compressed air and the flue gases. As highlighted previously, both subsystems share the same supply structure for economic reasons. The supply structure can commute when necessary and only one quantity can be supplied and only one variable can be controlled. Due to these properties, microalgae production process can be modeled as underactuated system with single input and multiple outputs (Freudenberg and Middleton 1999; Soria-López et al. 2013).

4.5 On/Off Controller

In the analysis presented in this chapter, the on/off controller is used as a reference control system, since it is the most commonly used controller in photobioreactor production process. The on/off controller is the most simple feedback regulator and it is suitable for the manipulated variable characterized by two states: open (on) and closed (off). The on/off regulator commutes the state of manipulated variable as a function of the set point and process output measurement. The most important drawbacks of this control technique are related to a poor disturbance rejection performance and oscillations around reference in controlled variables.

5 Control System Results

In this section, the results for practical evaluation of event-based control schemes for industrial photobioreactors are presented. This study considers three different configurations that are analyzed below.

5.1 Event-Based Controller pH in Tubular Photobioreactor

This section shows real experiments using the event-based control strategy applied to pH control in tubular photobioreactor described in Sect. 2.1. A preliminary simulation study on this event-based approach can be found in Pawlowski et al. (2014). Notice that in all considered control systems the continuous signal obtained from the controller must be translated into a discontinuous signal used to drive the valve. For this purpose, a PWM (Pulse Width Modulation) technique is used, with a frequency of 0.1 Hz. Notice that microalgae growth requires that the operation variables must be maintained at optimum values, where for the microalgae used in this evaluation, *Scenedesmus almeriensis*, an optimal value of $w_{pH} = 8$ is required.

As mentioned previously, the experiments are performed using the event-based GPC and the classical time-based GPC to control pH in an industrial tubular photobioreactor. The experiments have been performed between June 6 and 12, 2013, at the plant described in Sect. 2.1. During this period, the photobioreactor plant worked in the continuous mode. In this mode, 34% of the total volume of culture is harvested daily. The harvesting operation is done usually around midday and this influences on the pH value. From the control system point of view, this issue is considered as an unmeasurable disturbance, since the harvesting operation is performed by the plant operator to cover product demand. All control schemes are implemented on an industrial PC located at the plant facilities. The controller with Labview-based software executes the event-based GPC controller or classical GPC, which are coded in the Matlab environment. All control system sensors and actuators are connected to the Compact-FieldPoint unit from National Instruments. In such configuration, the controller node communicates with Compact-FieldPoint through a dedicated ethernet network to perform sensing and control tasks.

The first tested control scheme was the classical time-based GPC and was developed using model presented in Sect. 2.1.1. The control system configuration and algorithm tuning parameters are as follows: sampling time of 1 min was used with the following parameters: $N_u = 5$, $N_2 = 20$, and $\lambda = 0.005$. The value of the solar irradiance in the prediction horizon is considered constant and equals to the last measured value. This configuration was implemented and tested to assure the proper control system configuration and check the control performance in the presence of measurement noise and external disturbances that affect controlled variable. Prior to the controller implementation, several tests were made to confirm the proper model validation around the operation point. This procedure confirmed the relatively good accuracy of model (see Sect. 2.1.1), to guarantee the desired control performance.

The time-based GPC was tested during two different days and representative results of one of those days are shown in Fig. 9 (notice that classical GPC was already studied by the authors in the previous works and for that reason it was only used here for comparisons Berenguel et al. 2004). It can be observed how GPC controller regulates the pH of the photobioreactor compensating the influence of the solar irradiance (main control system disturbance). On the other hand, it can be observed how the controller reacts to the unmeasurable process disturbances (harvesting operation), between 12 and 15 h. It can be also seen that despite of the disturbance, the controller maintains the pH close to the set point, due to aggressive compensation of the control signal. During this day, the classical GPC obtains a good control accuracy, comparable with the results obtained through simulations, what was confirmed by IAE = 1357 index. The accumulated IT reaches 342 min that corresponds to 1262 g of pure CO_2. To achieve this performance, an amount of 350 g of CO_2 was lost. The microalgae growth performance indexes for RO_2 and P_b were 0.7509 [$kg_{[O_2]}/m^3$ day] and 0.5572 [$kg_{[b]}/m^3$ day], respectively.

For this particular day, the classical GPC obtains a good accuracy in pH regulation. However, this good control performance is obtained through a high CO_2 consumption, what also increases the CO_2 losses. This fact confirms the results obtained through simulations. As mentioned previously, the CO_2 loss is an important issue in

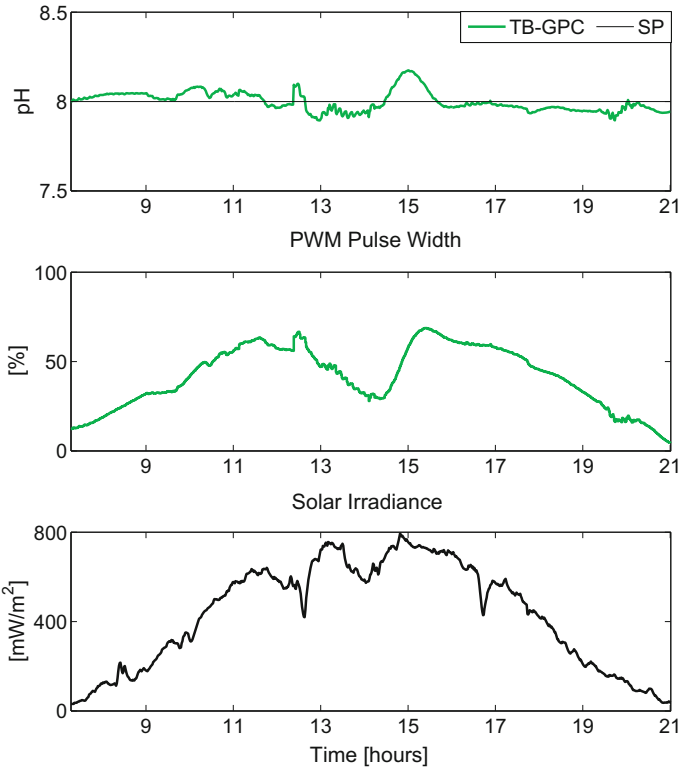


Fig. 9 Experiments control results for classical time-based GPC (Pawlowski et al. 2014)

photobioreactor pH control problem and any solution that faces this problem is welcomed. In such perspective, the event-based GPC arises as an interesting option to reduce the control effort at the expense of a reasonable control performance accuracy degradation.

The event-based GPC algorithm was implemented with the same configuration parameters as in the simulation study. Again, the control objective is to maintain the pH value close to the set point with the desired tolerance. Such a tolerance is determined by the sensor deadband value, selected to $\beta = 0.075$ for whole test period according to the relationship between the pH and the photosynthesis rate described in Sect. 3. The application of the event-based controller makes possible to establish the tradeoff between control performance accuracy and CO_2 losses.

The event-based configuration has been tested during 5 days and the overall control performance is shown in Fig. 10. The event-based GPC control structure with sensor deadband was implemented with $T_{base} = 1$ min, $T_{max} = 5$ min, $n_{max} = 5$, and thus $T_f \in [1, 2, 3, 4, 5]$. The control horizon was selected to $N_u^{n_{max}} = 5$ samples for all GPC controllers. The prediction horizon was set to $N_2^c = 20$ min in continuous time and was used to calculate the equivalent prediction horizon for each controller with

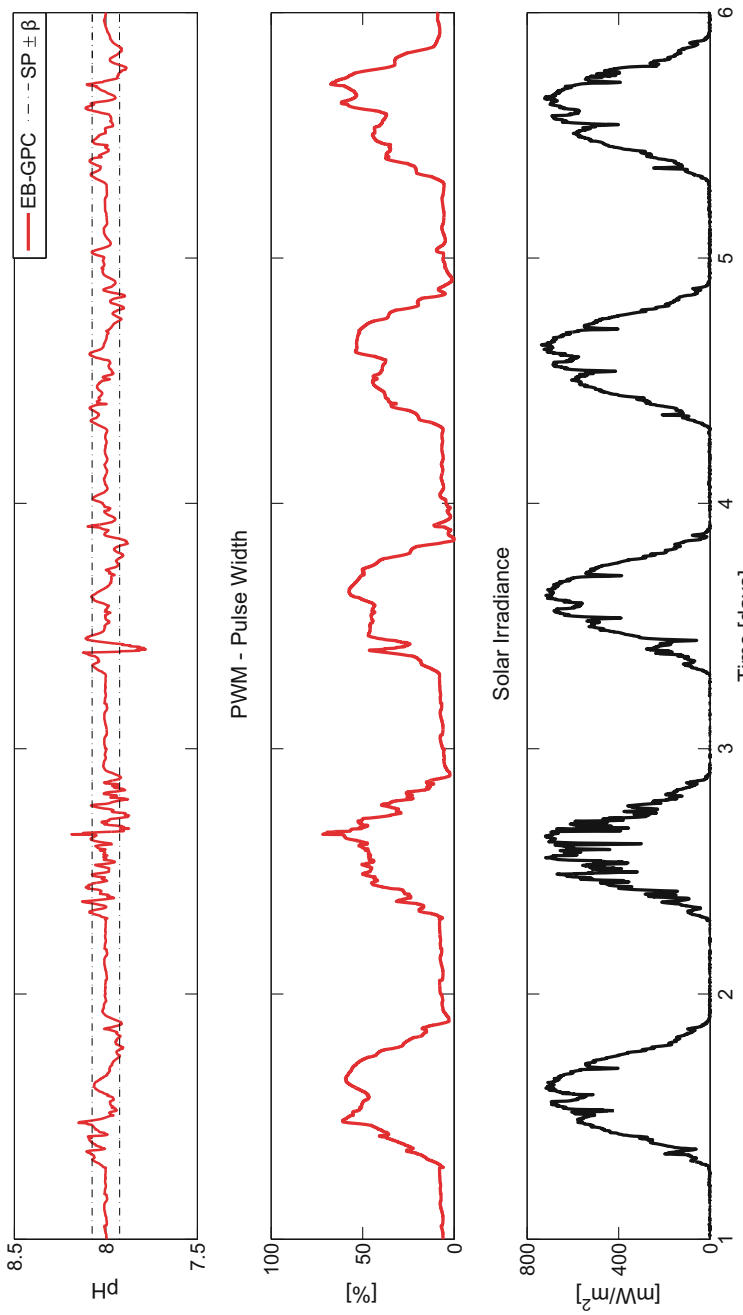


Fig. 10 Experimental event-based control results for a 5-day period (Pawlowski et al. 2014)

a different sampling period (Pawlowski et al. 2012b). Finally, the control weighting factor was adjusted to $\lambda^f = 0.005$ to achieve the desired closed-loop dynamics for the faster GPC controller with $T_1 = T_{base} = 1$ min. Due to the physical limitation of the actuator signal (PWM pulse width), the event-based GPC controller considers constraints on the control signal $0 < u(t) < 100\%$. Taking into account all design parameters, the control structure is composed of five controllers for each possible sampling frequency. Considering pH operating limits for tubular photobioreactor ($7 < \text{pH} < 9$) and the relationship of the pH with the photosynthesis rate (as will be described below), the β parameter was set to $\beta = 0.075$. In such a way, event-based configuration will keep pH close to its optimal value and allows to find an acceptable tradeoff between performance and control effort (Pawlowski et al. 2012b).

It can be observed that for most of the time the controlled variable is inside the established band SP $\pm\beta$. The pH goes outside the band only a few times during the whole test period and this fact is induced by the external disturbances. This is especially visible during the second and third days, where solar irradiance is affected by passing clouds, which changes the photosynthesis rates and affects the pH values. On the other hand, the obtained results show that the event-based controller properly rejects unmeasurable disturbances due to microalgae harvesting action. During clear days, the event-based controller maintains the pH inside the band, updating the process control action at the low rate (with T_{max} sampling time). When the pH crosses the sensor deadband limit, the control structure switches to the fastest sampling frequency T_{base} to move back controlled variable into the set point tolerance band.

Figure 11 shows a detailed view for the fourth day. It can be observed that the event-based GPC provides a good control accuracy. Moreover, it can be seen that each time the pH crosses the sensor deadband, the event-based control system increases the sampling frequency. Additionally, it can be observed how the load disturbance related to harvesting process is rejected properly by event-based controller around the midday. The event-based GPC controller generates less-aggressive control signal as long as the pH is inside the limits. When the controlled variable goes out the limits, the event-based scheme increases sampling frequency to compensate for the disturbances. The bottom graph in Fig. 11 shows how events are generated and can be seen that the event generation frequency increases when the pH goes outside the limit. On the other hand, the sampling frequency decreases when pH is within the limits. In this way, it is possible to reduce the controlled system updates. Hence, as a consequence of the reduced number of process updates, the event-based control system reduces the required control effort related to the CO_2 consumptions as well as CO_2 losses.

Table 1 collects the performance indexes for event-based GPC with sensor deadband in the experimental results. This table shows the Integrated Absolute Error (IAE), the number of events (Event), the Injection Time (IT) in minutes, and the total CO_2 supplied and the CO_2 losses, both expressed in grams. Moreover, two complementary indexes are calculated for oxygen and biomass production, RO_2 and P_b, respectively. Those indexes are used to evaluate analyzed control algorithm and its influence on microalgae culture. The first one is used to express the overall oxygen production rate that is influenced (among others) by solar irradiance and pH.

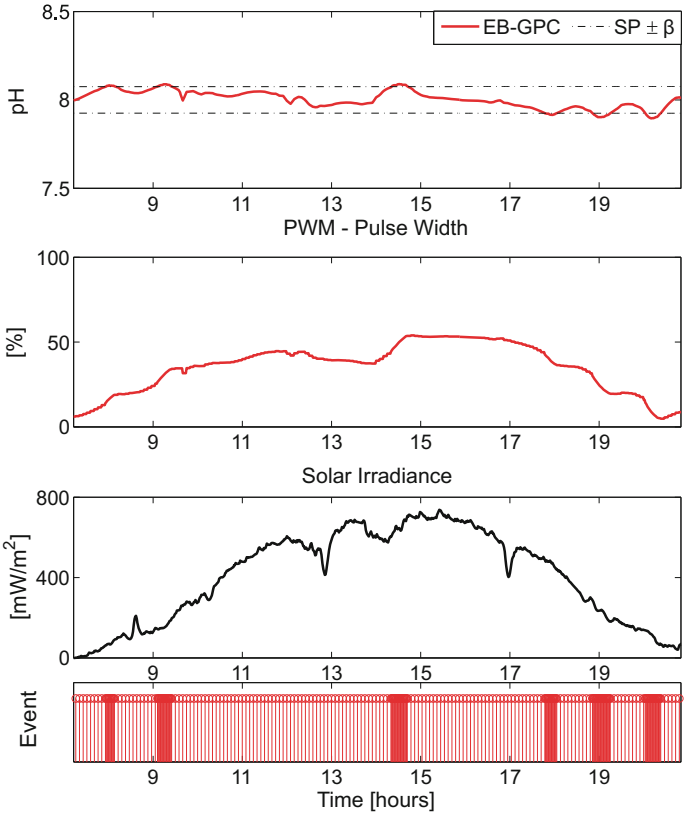


Fig. 11 Experimental control results for event-based GPC for the fourth day (Pawlowski et al. 2014)

Table 1 Event-based control system: performance indexes for the experimental results

		Day				
		1	2	3	4	5
EB-GPC	IAE	1725	1555	1666	1341	1579
	SLT (min)	767	767	767	767	767
	Event	214	280	269	228	258
	IT (min)	319.6	291.1	296.4	284.3	307.8
	CO_2 (g/day)	1177	1072	1091	1047	1134
	Lost CO_2 (g/day)	235	216	213	208	227
	RO_2 ($kg_{[O_2]}/m^3$ day)	0.8464	0.9953	0.9317	0.7526	0.7459
	P_b ($kg_{[b]}/m^3$ day)	0.6196	0.7445	0.6783	0.5532	0.5378

The second one shows the biomass production rate (daily measurements), which corresponds to reactor production performance.

It can be observed that the overall IAE index is slightly worst in comparison with those obtained through simulations. This is mainly due to harvesting process that affects the pH control accuracy and was not considered during the simulation study. On the other hand, one can observe how the event-based control scheme reduces the number of events in comparison to the SLT (Solar Light Time), which corresponds to the number of invocation of classical time-based control scheme. The CO_2 usage was similar to the values obtained during simulation study, which confirms the reduction of its usage with respect to the time-based scheme. This fact is also confirmed by the accumulated IT for all considered days. At the same time, it can be seen that the CO_2 losses are reduced. On the other hand, the microalgae growth indexes, RO_2 and P_b, were quite similar for the different days and are comparable with the classical GPC, analyzed previously.

All those benefits are obtained at expense of the control performance taking as a reference of the time-based GPC controller. Moreover, event-based configuration is able to reduce around 30% CO_2 losses obtained by classical GPC. Finally, notice that when event-based GPC controller is compared with on/off controllers (which are the type used in industrial photobioreactor), the control accuracy is highly improved and the CO_2 losses are reduced by more than 2 times.

5.2 Event-Based Controller pH in Raceway Photobioreactor with Efficient CO_2 Usage

The experimenters presented in this section have been performed during 2-week period, between January 27 and February 9, 2014. The analysis is divided into two sets that are related to event-based and on/off control approaches. During the first week, on/off controller was applied for pH control task. The second week was dedicated to event-based GPC evaluation. For both control techniques, the pH reference was set to $w_{pH} = 8$, being optimal for the grown microorganism. The raceway photobioreactor process setup used for this study is shown in Fig. 12. The main objective of these tests is to show the advantages that can be obtained with event-based control technique that focuses on the effective usage of flue gases. It has to be highlighted that the main motivation of this analysis is not a direct comparison of the introduced control techniques, since they are representing two extremely different degrees of complexities as well as different approaches.

5.2.1 On/Off Controller—pH Control Results

In the analyzed period, the pH control task was active only during diurnal periods, since photosynthesis process is active when solar irradiance is available. Moreover,

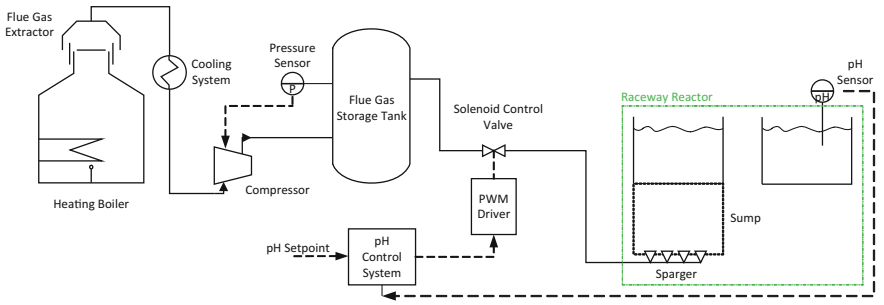


Fig. 12 Raceway reactor with pH control system using flue gases (Pawlowski et al. 2014)

the implementation details are as follows: sampling time was set to 1 min and the control signal is switched between 0 and 100% due to PWM technique. In Fig. 13, the on/off controller results for 7 days period are shown. From this result, it can be observed that on/off regulator maintains the pH level close to the reference. However, the obtained control performance has a low accuracy provoked by the significant pH oscillations (see the first plot in Fig. 13). This issue originates from abrupt changes in control variables (on–off actions). In analyzed configuration, the controller opens the solenoid control valve and applies some flue gases volume until the pH level decreases under selected reference. Once the controlled variable is in the desired range, the on/off controller closes the solenoid value, disabling injection of flue gases. Due to this working principle, a significant oscillation in controlled variable appears, which results in nonoptimal conditions for microalgae culture. The described behavior is present in whole analyzed period (bottom plot in Fig. 13), and appears independently on process disturbances (solar irradiance in this case).

From Fig. 14 (presenting detailed information for the third analyzed day), it can observe the low control performance mainly due to significant oscillations. The presence of these osculations came from two issues that are not considered by this simple controller. The first one is related to the process dynamics that is not considered in controller structure, and the second is due to plant's dead time (see Sect. 2.2.1 for details). Those two features of the pH control process result in large control actions that in consequence apply high volume of flue gases, which quickly decreases the pH level. Despite overabundance of CO_2 in cultivation medium, only small part is assimilated by the photosynthesis process. The unabsorbed carbon dioxide is emitted into atmosphere having negative effect on the environment. This issue is of high importance for large-scale industrial photobioreactors, since huge volume of flue gases is needed for the pH control purposes.

Control performance measures for the on/off controller are summarized in Table 2. These measures include the injection time (IT) expressed in minutes, the Integrated Absolute Error (IAE), and the total amount of flue gases (Gas) injected to the raceway photobioreactor. Additionally, the complementary measures related to production rate are computed and contain oxygen production RO_2, biomass concentration C_b expressed in grams per liter (higher value signify better growth rate), and biomass

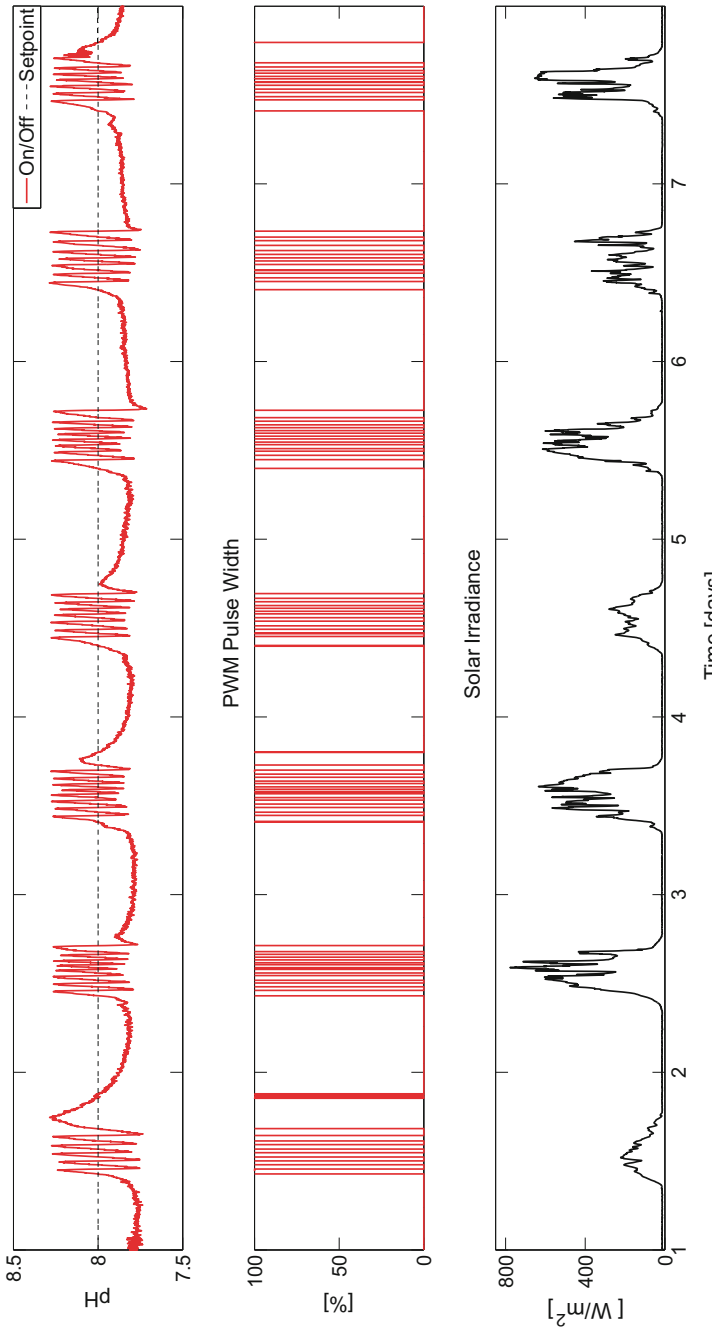


Fig. 13 On/off controller experimental results for a 1-week period (Pawlowski et al. 2014)

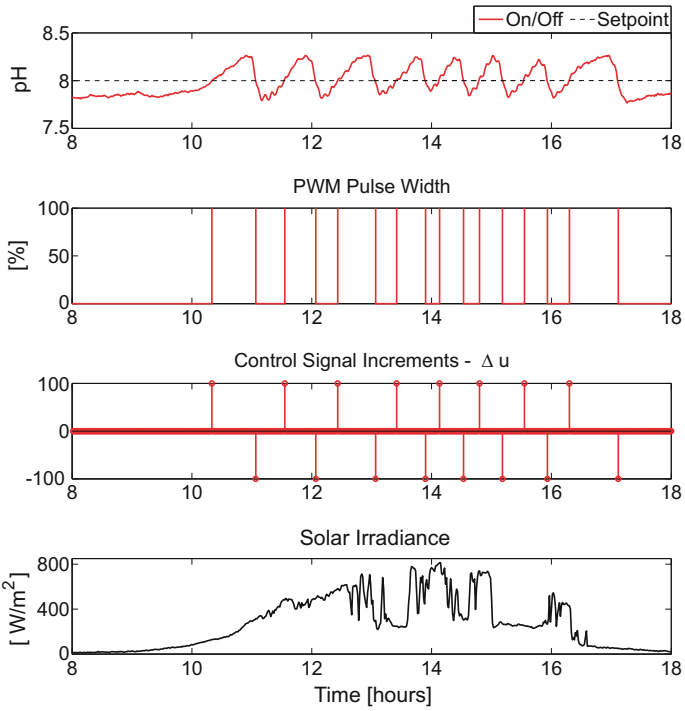


Fig. 14 Experimental results for the third day and on/off controller (Pawlowski et al. 2014)

Table 2 Performance indexes for on/off controller

Day	1	2	3	4	5	6	7
IAE	4503	4232	3436	3749	3945	4158	3678
IT (min)	293	291	298	279	299	290	316
Gas (m^3)	29.3	29.1	29.8	27.9	29.9	29	31.6
RO_2 (g/m^2 day)	7.4	6.8	7	7.2	6.9	7	7.1
C_b (g/l)	0.279	0.240	0.248	0.237	0.238	0.230	0.230
P_b (g/m^2 day)	6.2	4.83	5.2	5.7	4.76	4.68	4.68

production P_b (daily measure of the overall performance of the reactor). The RO_2 is computed using dissolved oxygen concentration measure (this index depends on solar irradiance and the pH value) and last two are based on laboratory analysis (Mendoza et al. 2013b). Such indexes are used to show the effect of tested algorithm on microorganism growth performance.

From the obtained values for the IAE index, it can be seen that on/off controller provides low accuracy for whole analyzed period. This is due to significant error between the pH value and the established set point. The IT measure indicates the

average injection time and reaches 280 min per day. The remaining indexes that characterize microalgae growth show an average production rate for this specific year season and on/off controller. Notice that all those measures will be compared with the results obtained using actuator virtual deadband and event-based GPC.

On the other hand, when continuous injection of flue gases is contrasted with on/off controller, the results are more than satisfied. Even this simple control strategy provides on-demand injections reducing volume of supplied flue gases, simultaneously keeping the pH value around the optimal level for culture growth. The atmosphere contamination is considerably lower, since the average volume of supplied flue gases is reduced to about 60%. Based on those indicators, the on/off controller gives a simple solution to decrease the carbon dioxide losses, as well as roughly to regulate the pH value.

Nevertheless, the results obtained with on/off controller can be considerably improved, when advanced control techniques, such as event-based predictive control system, are applied.

5.2.2 Event-Based GPC for pH Control

The event-based scheme was developed by following the methodology shown in Sects. 4.1 and 4.3. The design parameters for corresponding GPC controllers were set up as follows: the control horizon $N_2 = 20$ (capturing main process dynamics), the prediction horizon $N_u = 5$, the weighting factor for control signal $\lambda = 0.05$, and sampling time was set to 60 s. Moreover, to show the influence of actuator virtual deadband on the controlled process, two different values were evaluated. This parameter defines the tradeoff between control accuracy and control effort as well as overall control system performance. Considering these features, the virtual deadbands were established to $\beta_u = 1.5\%$ and $\beta_u = 1\%$ to satisfy control system goals. Additionally, all event-based GPC controllers were implemented with constraints on control signal ($0 < u(t) < 100\%$), due to the limitations defined by the PWM technique.

The introduced event-based GPC was tested during 1-week period on the industrial-scale raceway photobioreactor, and the results are presented in Fig. 15. Analyzing the pH variable, it can be observed that event-based controller improves significantly the control accuracy, when compared to the on/off control strategy. In this case, the pH slowly changes its value and it is kept arrowed the set point with better accuracy. The slow changes are provoked by the solar irradiance that affects the photosynthesis process (increasing the oxygen production), which forces the variations in the pH value. Due to these properties, the solar radiation is treated as control system disturbance and it is handled properly by the event-based controller. Its influence is compensated by controller through control signal increments. On the other hand, the microalgae production process is affected by the harvesting procedure. This action is performed at the midday and can be considered as unmeasurable load disturbance. Despite strong influence on the reactor parameters, the event-based GPC is able to keep the pH level close to established set point.

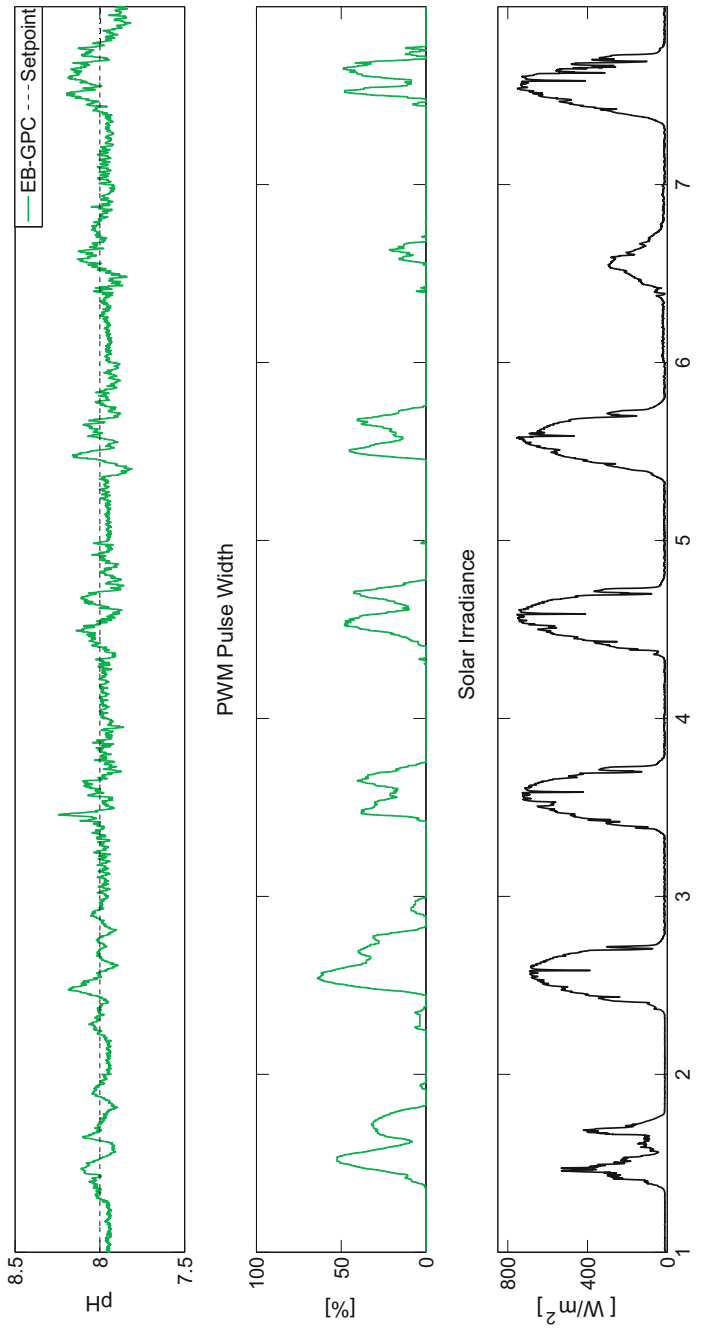


Fig. 15 The EB-GPC controller experimental results for a 1-week period (Pawlowski et al. 2014)

When control results for on/off controller and event-based GPC (shown in Figs. 13 and 15, respectively) are compared, it can be observed that event-based GPC improves control performance (as expected), mainly due to less-aggressive changes in control signal. The control signal provided by the event-based GPC (second plot in Fig. 15) is more smoother and changes gradually counteracting to disturbance dynamics. Moreover, the presence of actuator virtual deadband forces the control signal increments to meet the established minimum and suppressing the small changes, which result in an efficient use of resources. The established deadband is considered in the optimization procedure providing control signal increments bigger than the selected deadband. Due to this feature, it is possible to manage efficiently the volume of flue gases injected to the raceway photobioreactor.

The detailed view for the analyzed event-based controller (for the third day) is shown in Fig. 16. This specific day is characterized by very small variation of the pH (average deviation is less than $\pm$ 0.1 from the set point), which creates optimal conditions for microorganism growth. From the control signal (second subplot in Fig. 16), it can be seen how the event-based GPC compensates the photosynthesis effect that influences the pH value. Additionally, the volume of injected flue gases is strictly limited to cover actual demand. Considering this operation mode, it is possi-

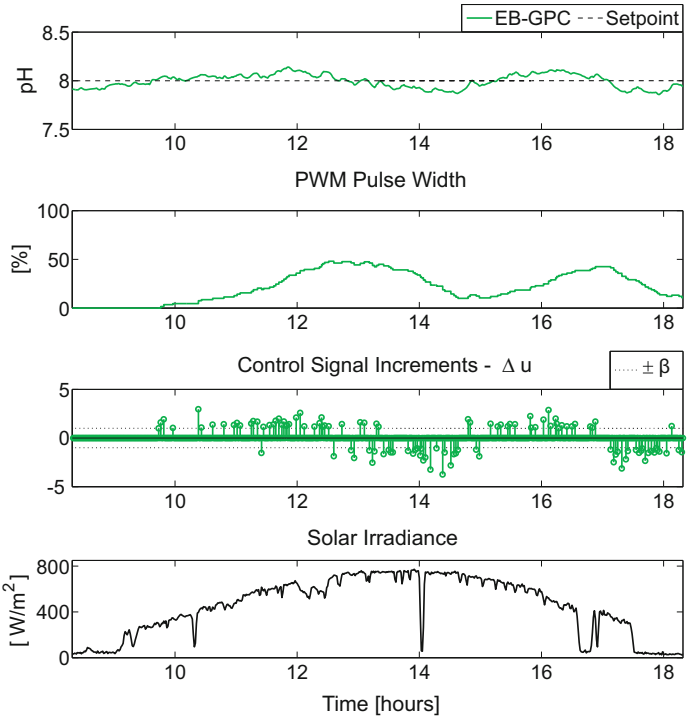


Fig. 16 The EB-GPC controller experimental results for the third day (Pawlowski et al. 2014)

ble to decrease the overall amount of flue gases used for control task, when compared to on/off regulator. The evolution of control signal increments is shown in the third plot, where also deadband value is highlighted. From this plot, it can be observed that all changes in control action are generated outside the established deadband. In consequence, the control system is less sensitive to insignificant error changes that require continuous control signal adjustments. Due to this feature, the event-based controller is able to establish the compromise between control resource utilization and control performance. From the microalgae cultivation process point of view, this property allows efficient use of the flue gases used for control task. In analyzed control scheme, the actuator virtual deadband β_u is used as an additional parameter that needs to be properly adjusted during controller design stage. Such a parameter should be set up taking into account the compromise in resource consumption and control performance. When deadband is set up to small value, the control accuracy is improved at the expense of resource utilization. In the opposite case, setting up the deadband to high value, it is possible to decrease the control resource utilization, which results in lower control accuracy.

Table 3 summarizes the control performance measures for event-based GPC evaluation. As mentioned previously, two different deadband values were used to test its influence. During first 2 days, $\beta_u = 1$ were used, and afterward its value was changed to 1.5. The computed IAE values for first 2 days are lower when compared to the days with $\beta_u = 1.5$ (verifying previously features for event-based controller). Independent on the deadband value, the IAE index is significantly improved in comparison to the on/off regulator, gaining about 50% in control system accuracy. Moreover, the average value of IT index was reduced over 40%, and simultaneously the amount of the used flue gases is also minimized. Taking into account these features, the event-based control scheme allows to improve control performance and reduce the volume of the flue gases used for control purposes. The last feature is of high importance for large-scale photobioreactors, since the volume of carbon dioxide wastage is minimized. As a consequence, event-based controller prevents the emission of important volume of greenhouse gases into atmosphere. It should be highlighted that the event-based control approach improves the CO_2 fixation through the efficient management of flue gases.

Table 3 Performance indexes for event-based GPC controller

Day	1	2	3	4	5	6	7
β_u [%]	1	1	1.5	1.5	1.5	1.5	1.5
IAE	1525	1661	1817	2239	2098	1569	2241
IT (min)	222	269	189	183	159	82	165
Gas (m^3)	22.2	26.9	18.9	18.3	15.9	8.2	16.5
RO_2 (g/m^2 day)	10.6	11.7	12.5	10.6	13.3	11.5	14.8
C_b (g/l)	0.329	0.321	0.341	0.349	0.384	0.367	0.395
P_b (g/m^2 day)	7.85	7.19	8.14	6.50	8.22	7.36	8.85

On the other hand, the improved control system performance has a beneficial influence on the microorganism growth rate. All related measures (RO_2, C_b, and P_b) were improved. The average RO_2 value increased about 50% in comparison to its average value for on/off control technique. In the case of biomass concentration, this index was improved around 30%. The P_b measure also gets better obtaining average value of 7.6 g/m^2 day. Considering all these results, it is demonstrated that evaluated event-based controller allows to improve the pH control accuracy in race reactor (keeping the optimal cultivation conditions) and also provides the effective usage of flue gases.

5.3 *Selective Event-Based Controller for pH and Dissolved Oxygen in Raceway Photobioreactor*

In this section, the evaluation of the selective event-based control system is presented. Resulting raceway phitobioreactor setup for such a scheme is shown in Fig. 17, where the pH process is controlled with flue gases and dissolved oxygen is regulated using compressed air.

The experimental evaluation was performed for 1-week period (from July 20 to July 27, 2014). Selected time period allows us to test the implemented selective event-based scheme during diverse solar irradiance profiles, providing reliable results. The control scheme was developed using the approach presented in Sect. 4.4. In this scheme, the pH process is controlled by event-based GPC and dissolved oxygen is regulated by on/off control technique. The event-based GPC relays on process model and the first step in control system development was devoted to capture the dynamics between the pH value and carbon dioxide injections. Once the process model was determined (see Sect. 4.4), the control structure was ported to raceway reactor SCADA system.

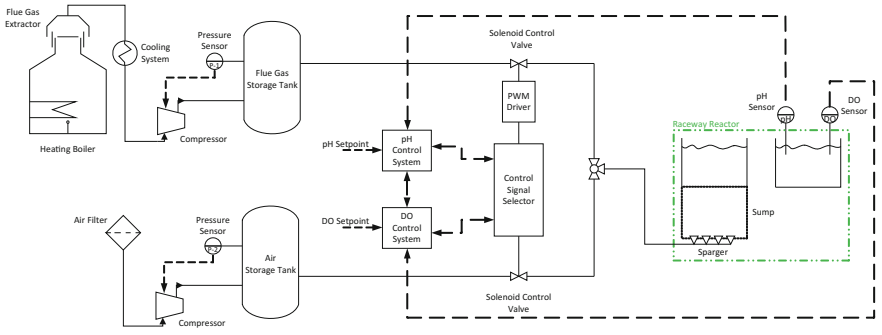


Fig. 17 Selective control scheme: raceway reactor setup for the pH and dissolved oxygen control (Pawlowski and Mendoza 2015)

The GPC structure was implemented with the following setup: the prediction horizon was set to $N_2 = 20$, the control horizon N_u was set to 5, and the control signal weighting factor $\lambda = 0.05$. The sampling time was established to 1 min, considering process dynamics. Moreover, following the results from (Pawlowski et al. 2014), the actuator virtual deadband β_u was set to 1.5. Due to the PWM technique, the GPC optimization procedure includes the constraints on control signal ($u_{pH} \in 0{-}100\%$). As shown in the previous section, the actuator deadband provides the possibility to establish the tradeoff between control system accuracy and control system effort (flue gas usage in this case). For this study, the pH reference was set to 7.7 and for the dissolved oxygen, set point was selected to 150 [%Sat]. The dissolved oxygen was controlled with on/off technique (with 1 min sampling time) provided on-demand injection of compressed air.

Regarding the selective control parameters, the switching condition (see Eq. 12) was implemented using $SCSP_{pH} = 8$, with tolerance $\pm\beta = 0.3$ and reference for dissolved oxygen was set up to $SCSP_{DO} = 200$ [%Sat]. Following the selective control working principle, the pH value will be kept within set point ± 0.3 interval and this tolerance will enable the possibility to maintain the dissolved oxygen concentration under the dangerous value. In such a case, the dissolved oxygen control task has a lower priority and is executed only when the pH level is inside the selected limits. As shown previously, the provided tolerance has an insignificant influence on photosynthesis rate and, in this approach, is exploited to provide dissolved oxygen control. Being an important advantage, the oxygen concentration is kept under the limit, which in consequence improves the photosynthesis rate and volume of biomass production.

The results obtained from the analyzed system are shown in Fig. 18. From the first plot, it can be observed that pH level is controlled within the established limit and only during nocturnal periods drop under the band (photosynthesis process is not active during the night and no pH control is performed). Additionally, it can be seen how the event-based GPC handles the changes in solar irradiance (main control system disturbance), which is reflected in the control signal that captures variations in solar irradiance (Fig. 18). Moreover, from the pH control signal, it can be observed how the switching mechanism operates. In certain situations, the pH control signal is switched to zero and then controller for dissolved oxygen is activated. This is due to raceway reactor shared by actuation structure, which can change the injected gas type (atmospheric air or flue gases). The swapping action between two controllers is activated when the event-based triggering condition meets the logical sequence (Eq. 12 in Sect. 4.4). Due to this working principle, two controllers can be executed in parallel, meeting diverse control system objects that can be with classic approach. This operation mode is confirmed by the changes in control for pH and dissolved oxygen (second and fourth plots, respectively).

The control results for dissolved oxygen control subsystem are shown in third and fourth plots in Fig. 18. For this specific variable, the on/off controller provides compressed air injections to reduce the oxygen concentration. The obtained results show the efficacy of devolved control scheme, since the dissolved oxygen is kept below the established limit (200 [%Sat]). It should be highlighted that dissolved oxygen

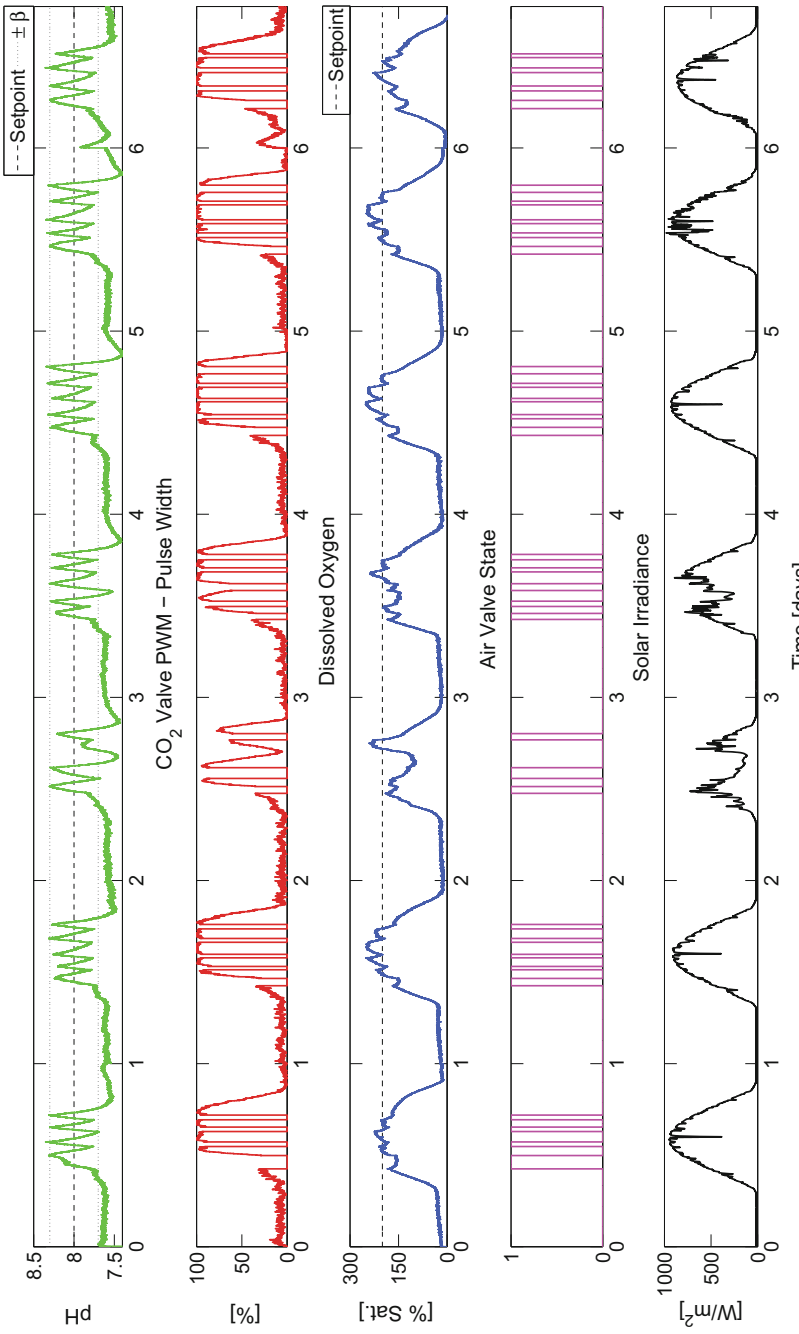


Fig. 18 Experimental results of the selective control strategy with the event-based approach for pH and dissolved oxygen control (Pawlowski and Mendoza 2015)

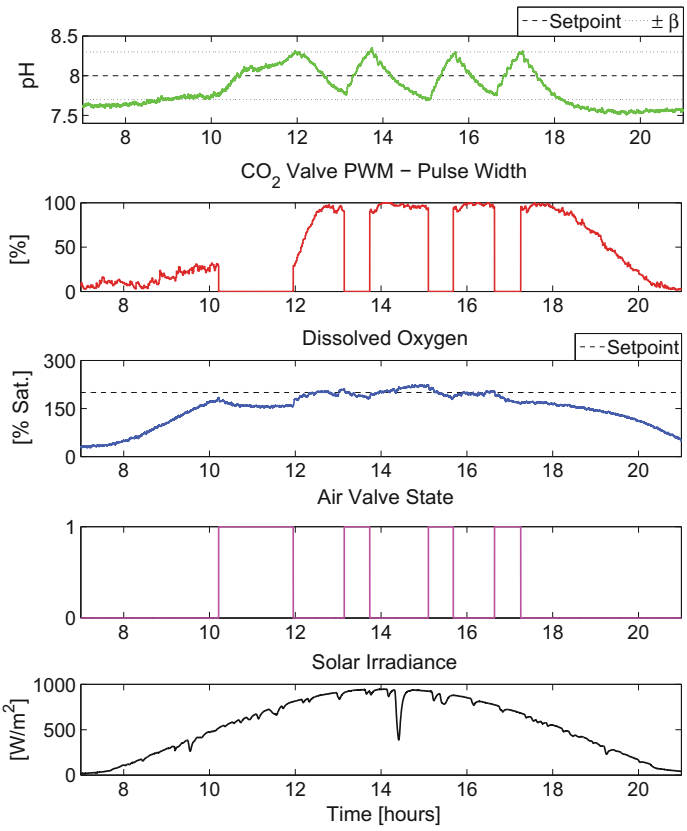


Fig. 19 Selective control details for the first day—solar irradiance without clouds (Pawlowski and Mendoza 2015)

concentration rarely exceeds the 250 [%Sat], which is marked as the dangerous limit. This situation is beneficial for the microalgae growth, since the cultivation process is performed in optimal conditions.

The day presented in Fig. 19 is characterized by stable solar irradiance, which stimulates the photosynthesis process. From the first plot, it can be observed that the pH value is maintained inside the tolerance band despite high solar irradiance. Due to high photosynthesis rate, the event-based GPC provides a high amount of flue gases to deliver necessary carbon dioxide and to reduce the pH level. During this specific day, the control system operates close to the upper saturation limit. From the dissolved oxygen point of view, its value is kept below the critical value for most of the day. Nevertheless, during central hours of the day, its value exceeds for few minutes above this established limit. This is due to the photosynthesis process peaked that results in high dissolved oxygen concentration. The main reason of this excess is due to the pH prioritization, since this parameter is critical in cultivation process.

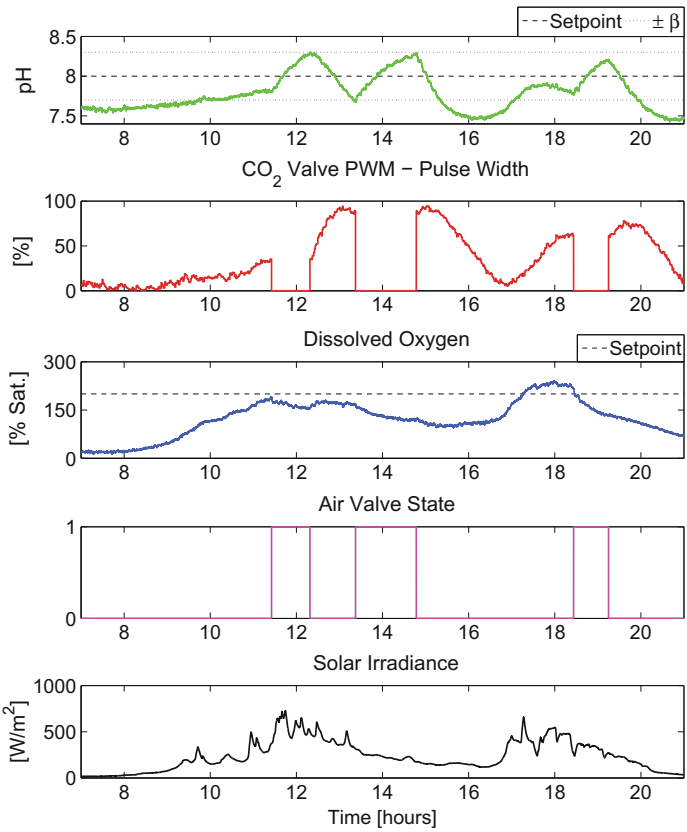


Fig. 20 Selective control details for the third day—solar irradiance with passing clouds (Pawlowski and Mendoza 2015)

Despite this insignificant issue, both variables are close to the optimal values, thanks to the selective event-based control approach.

The second analyzed day (see Fig. 20) is characterized by strong variations in solar irradiance, due to passing clouds, and is used to show how the event-based selective control reacts to disturbances. From such figure, it can be seen that until 12 am control system provides good control accuracy for both variables. This situation changes between 14 and 16 h, since the system perturbation (solar irradiance) abruptly changes the value affecting the microorganism production rate. In consequence, the pH value moves outside the selected band. However, the event-based GPC reacts decreasing the volume of injected flue gases in order to increase the pH. During this action, the selective controller focuses on the pH value (being more important) and once recovered its optimal value also handles the dissolved oxygen. Moreover, from the control signal for the dissolved oxygen, it can be observed that compressed air is provided to the raceway photobioreactor only when necessary.

Table 4 Performance indexes for the selective control strategy with the event-based approach

Day	1	2	3	4	5	6	7
IAE_{pH}	108	84	118	97	80	83	95
IT (min)	298	352	194	314	342	340	342
Gas (m^3)	29.8	35.2	19.4	31.4	34.2	34.0	34.2
IAE_{DO}	1441	1601	2969	1791	1620	1693	2270
DO_{OLT} (min)	111	264	64	65	259	254	56
RO_2 (g/m^2 day)	25.7	25.8	32.4	30.7	29.9	28.8	28.3
C_b (g/l)	0.57	0.74	0.64	0.63	0.62	0.63	0.63
P_b (g/m^2 day)	17.5	20.6	23.5	17.4	20.2	19.4	18.7

This feature is important from the entomic point of view, since it allows us reducing maintenance costs when compared to the system with continuous aeration system.

The control performance indexes for analyzed scheme are summarized in Table 4. These measures include IAE_{DO} and IAE_{pH}—integrated absolute errors (for pH and dissolved oxygen, respectively), IT—the injection time in minutes, Gas—volume of flue gases injected to the photobioreactor, and DO_{OLT}—the amount of time when dissolved oxygen measure is over the limit (200 [%Sat]). Additionally, three measures related to the microalgae production were computed: RO_2—oxygen production, P_b—biomass production, and C_b—biomass concentration expressed in grams per liter, respectively. The RO_2 index is computed using the dissolved oxygen measures and it is proportional to photosynthesis rate (Mendoza et al. 2013b). The last two were obtained from laboratory study. All these indexes are used to the influence of event-based selective control on the microorganism growth.

Analyzing the IAE measures, it can be observed that control accuracy is significantly higher for pH variable. This is due to selective control configuration, where the pH is prioritized over the dissolved oxygen. Additionally, the event-based GPC scheme is able to use the control resources (flue gases in this case), minimizing the overall supplied volume (see IT measure). Nevertheless, this control performance is lower when compared to the classical control approach that handles the pH individually. On the other hand, the selective control system is able to control both parameters at the expense of control accuracy deterioration. This operation mode allows us to establish the tradeoff between the pH and dissolved oxygen control accuracy and can be exploited to simultaneous control of both variables. Due to these properties, it is demonstrated that simple on/off regulator can maintain oxygen concentration under the danger limit. The DO_{OLT} index shows that the dissolved oxygen is inside the safe zone and rarely exceed the 250 [%Sat]. Another important feature of event-based selective control is related to energy savings in aeration system, since the compressed air is applied when strictly necessary. The results provided for industrial-scale reactor are satisfactory, since both variables are kept around their optimal values. Moreover, the analyzed control system is able to address several issues related to maintenance costs and efficient use of control resources, using standard reactor architecture.

Taking into account these features, the event-based selective control scheme provides a unified solution for simultaneous control of the pH and dissolved oxygen.

The microorganism cultivation indexes indicate very good growth conditions due to the application of event-based selective control approach. It needs to be mentioned that the average biomass production rate (P_b) and biomass concentration show C_b for common control approach (on/off regulator for pH variable) are around 17 and 0.57, respectively, whereas the analyzed control technique improved the overall photobioreactor productivity around 15% (average value for all indexes) for 1-week period. Notice that these results can be extrapolated for large-scale photobioreactors and result in increased productivity of raceway reactor.

6 Summary

In this chapter, the practical evaluation of event-based control schemes for industrial photobioreactors was analyzed. This study considers three different configurations that were implemented for control tasks in microalgae culture process.

In the first analyzed configuration, an event-based GPC control strategy has been used to reduce CO_2 losses in pH control of tubular photobioreactors by keeping an adequate control performance level. The core idea consists of updating the controlled process only when significant deviations from the set point occur. The presented control scheme provides savings in plant maintenance cost, minimizing the CO_2 losses. The obtained benefits are reached at the expense of control performance through control accuracy degradation. The desired tradeoff between control performance and CO_2 losses can be easily implemented adjusting a sensor deadband value at the control system design stage. The experiments show that the event-based GPC control scheme is suitable for pH control in tubular photobioreactors. The obtained reductions in CO_2 losses are even more significant than in the case of classical GPC analyzed in Berenguel et al. (2004). This is due to the event-based framework, which allows to reduce the resource utilization at expense of the control performance. In such case, it is possible to meet the compromise between control accuracy degradation and plant maintenance costs as well as environmental impact. The evaluation performed in this study is based on a single photobioreactor, but it can be easily extrapolated to industrial scale. In such case, the benefits are even more visible.

The second analyzed event-based control scheme provides interesting results on the efficient use of the flue gases in microalgae cultivation process. The event-based GPC with actuator virtual deadband allows to decrease the volume of injected flue gases into raceway photobioreactor when collated with results for commonly used controller. Performed evaluation demonstrated that the use of event-based approach in raceway reactor process improves the growth rate, since the cultivation conditions are improved through better control accuracy. Additionally, tested configurations supply significantly smaller flue gases volume due to its efficient management in controller task.

The last studied event-based approach addresses the pH and dissolved oxygen control problems simultaneously using a unified structure. To this end, the event-based selective control scheme is applied to raceway photobioreactor. The results of the experimental evaluation show that implemented control algorithm is able to handle both variables providing good control performance. Due to event-based system properties, the analyzed control scheme is able to adapt dynamically to the photosynthesis rate. Additionally, this property provides the mechanism to detect changes in the process state and is used as a trigger to switch between dissolved oxygen and pH controllers. In tested approach, the event-based GPC uses efficiently the flue gases (CO_2 source) for pH control and an on/off regulator reduces energy usage providing demand compressed air injections (for dissolved oxygen control). The application of event-based control scheme increases the microalgae production performance, since the growth parameters were kept close to its optimal values and the selective scheme allows us the optimization of the raceway production process.

From the obtained results, it can be seen that all evaluated event-based configurations improve the control accuracy (when compared to the commonly used controllers), while preserving the control resources. Taking into account all features and properties of tested event-based approaches, a great potential to improve the production rate is observed. However, the future effort for long-term evaluation as well as theoretical studies is required to make the event-based control approach widely accepted in the bioprocess industry.

Acknowledgements This work has been partially funded by the following projects: DPI2014-55932-C2-1-R, DPI2014-55932-C2-2-R, DPI2014-56364-C2-1-R, and DPI2012-31303 (financed by the Spanish Ministry of Economy and Competitiveness and EU-ERDF funds); Controlcrop P10-TEP-6174 (financed by the Consejería de Economía, Innovación y Ciencia de la Junta de Andalucía); and the UNED through a postdoctoral scholarship.

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Dynamic Modeling of Microalgal Production in Photobioreactors

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Abstract In this chapter, dynamic models for microalgal production in open and closed photobioreactors are presented. These models are first principle-based models, which take into account both spatial and temporal gradients for the main culture variables. Both fluid dynamics and biological phenomena are considered in the model equations. Calibration and validation tests are summarized in real open and closed tubular industrial photobioreactors, obtaining successful results. Finally, in view of the obtained results, conclusions about the capabilities of the developed models are drawn, as well as its main uses and applications.

1 Introduction

Microalgae production systems are being globally studied due to their high potential in different industrial fields. Microalgae can be used to develop bioproducts such as pharmaceuticals, cosmetics, animal feeds, etc. (Koller et al. 2014; Spolaore et al. 2006). Furthermore, since microalgae have a high combustion power, they have been classified as the third-generation biofuels, belonging to the renewable energy framework. On the other hand, thanks to CO_2 fixation that is performed by their cells during the photosynthetic process, these production systems allow to mitigate the greenhouse gases emission generated by other industrial processes, and they can be

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B.N. Tripathi and D. Kumar (eds.), *Prospects and Challenges in Algal Biotechnology*, DOI 10.1007/978-981-10-1950-0_2

even used in wastewater treatment processes. Usually, two types of photobioreactors are mainly used to produce microalgae: (i) closed photobioreactors as tubular or flat panels reactors, in which high-value products are produced by strains highly sensitive to contamination; and (ii) open reactors as open ponds and raceway reactors, simpler and less-expensive ones where contamination-proof strains can be produced (Acién et al. 2013; Posten 2009).

Nowadays, an important effort is being performed to introduce this microalgae-based technology in the energy market. Nevertheless, it is necessary to reduce costs and to guarantee that the production from the microalgae culture is performed in a controlled way with the best efficiency as possible. Therefore, the biomass production must be increased while at the same time the associated cost must be reduced in large-scale facilities. Optimization methodologies based on modeling and control approaches are becoming the solution to reach these objectives (Andrade et al. 2016).

Currently, from an automatic control and engineering point of view, a large number of applications related to the optimal production of biomass for systems based on microalgae are opened, owing to the lack for this kind of works in the literature (Andrade et al. 2016; Bernard 2011). The main reason of this lack is the absence of dynamic models that describe in an appropriate way the whole phenomena related to the growth and the biomass production. On the other hand, there are few studies related to the culture control conditions or addressed to the global optimization of these production systems from a control point of view (Andrade et al. 2016; Bernard 2011), mainly due to the high complexity of the required models or problems associated with the systems. For all these reasons, the availability of high-quality models for photobioreactors plays a key role in the control design stage for the optimization of the biomass production (Berenguel et al. 2004; García et al. 2003).

Nonlinear dynamic models are rarely found to represent the microalgal production processes based on photobioreactors. The main reason is because microalgae are photosynthetic organisms that are difficult to manage and use as they have a strong aptitude to store nutrients. Second, their pigments attenuate the light, which is their source of energy and this generates a strong coupling between biology (microalgae growth) and physics (radiative transfer properties and hydrodynamics). Finally, such organisms are most of the time far from the classical hypotheses (namely balanced growth) required to apply classical results in metabolic engineering. For that reason, most existing models describe separately some of these processes (Acién et al. 1998; Concasa et al. 2010), or considering steady-state balances where the reactor has been analyzed as a stirred tank reactor (Guterman et al. 1990; James and Boriah 2010; Jupsin et al. 2003; Xin et al. 2010).

Therefore, dynamic models that take into account the temporal–spatial distribution of culture parameters are necessary to adequately simulate this type of reactors. Moreover, these dynamic models are necessary to optimize the design and operation of the systems, helping to understand the different dynamics and phenomena taking place. Furthermore, these models can be used as predictive and simulation tools in order to properly design and operate these systems, as well as to design control

strategies for optimal biomass production such as pointed out above (Acién et al. 2013; Norsker et al. 2011).

This chapter presents two dynamic model of microalgae production in both tubular and raceway reactors (Fernández et al. 2014, 2016). The models are based on mass balances, transport phenomena, thermodynamic relationships, and biological phenomena taking place in the reactors, thus being based on fundamental principles instead of empirical equations. They take into account the kinetics of different phenomena inside the reactor, and thus a complete dynamic simulation model can be obtained. The models allow predicting the evolution of the main variables of the system such as biomass concentration, pH, dissolved oxygen, and total inorganic carbon in the liquid phase, in addition to oxygen and carbon dioxide exchange for the gas phase. Both models were calibrated and validated using experimental data from pilot-scale industrial reactors, resulting in powerful tools for the optimization of design/operation of this type of photobioreactors as well as for control design purposes.

2 Materials and Methods

This section summarizes the facilities and materials used for the experiments presented in this chapter.

2.1 Closed Tubular Photobioreactor

2.1.1 Microorganism and Culture Medium

The strain selected to be cultivated into the tubular photobioreactor was *Scenedesmus almeriensis* (CCAP 276/24, Culture collection of Algae and Protozoa of the Center for Hydrology and Ecology, Ambleside, UK). This strain stands temperature up to 45 °C and pH values up to 10, being its optimum conditions of 35 °C and pH 8 (Sánchez et al. 2008a, b). The experiments performed in this work took place in a tubular photobioreactor manipulated in continuous mode at a dilution rate of 0.34 1 day^{-1}. The culture medium was Mann & Myers, prepared using agricultural fertilizers instead of pure chemicals. The microalgae were grown photoautotrophically with a continuous aeration to avoid dissolved oxygen accumulation, under pH and temperature-controlled conditions.

2.1.2 Tubular Reactor and Operation Conditions

Experiments were performed on a tubular photobioreactor which belongs to a microalgal production facility, which is situated inside a greenhouse and located at research center “Estación Experimental Las Palmerillas”, property of CAJAMAR Foundation (Almería, Spain). Ten tubular fence-type photobioreactors were built as

described in Acién et al. (2001) and Molina et al. (2001). Figure 1 shows a view of this facility. The photobioreactor can be divided into two main parts (see Fig. 2). On one hand, the solar receiver is designed to maximize the interception of solar radiation, minimizing resistance to flow, and occupying the minimum area as much as possible. On the other hand, a bubble column is used for mixing, degassing, and heat exchange culture. The total culture volume is 2600 l; the photobioreactor has 19.0 m length and 0.7 m width. The solar receiver is made of transparent tubes joined into a loop configuration to obtain a total horizontal length of 400 and 0.09 m diameters. The microalgal culture is circulated at 1 m s^{-1} using a centrifugal pump located between the bubble column and the solar receiver. The pH of the culture is controlled by on-demand injection of pure CO_2 at 5 l min^{-1}. The bubble column has 3.2 m height and 0.4 m inner diameter, and the dissolved oxygen is removed by a constant airflow rate of 140 l min^{-1}. Furthermore, the culture temperature is controlled through an internal heat exchanger located at the bubble column by passing cooling water at 1500 l h^{-1}. The culture is harvested at an overflow at the top of the column when freshwater is poured into the bubble column. Moreover, the pH, temperature, and dissolved oxygen are measured at several positions (3 for dissolved oxygen: at the bottom, middle, and top of the photobioreactor; and 5 for pH and temperature from the bottom to the top of the photobioreactor, being evenly distributed) along the tube using Crison probes (Crison Instru-

Fig. 1 Real view of the tubular photobioreactor at the experimental station

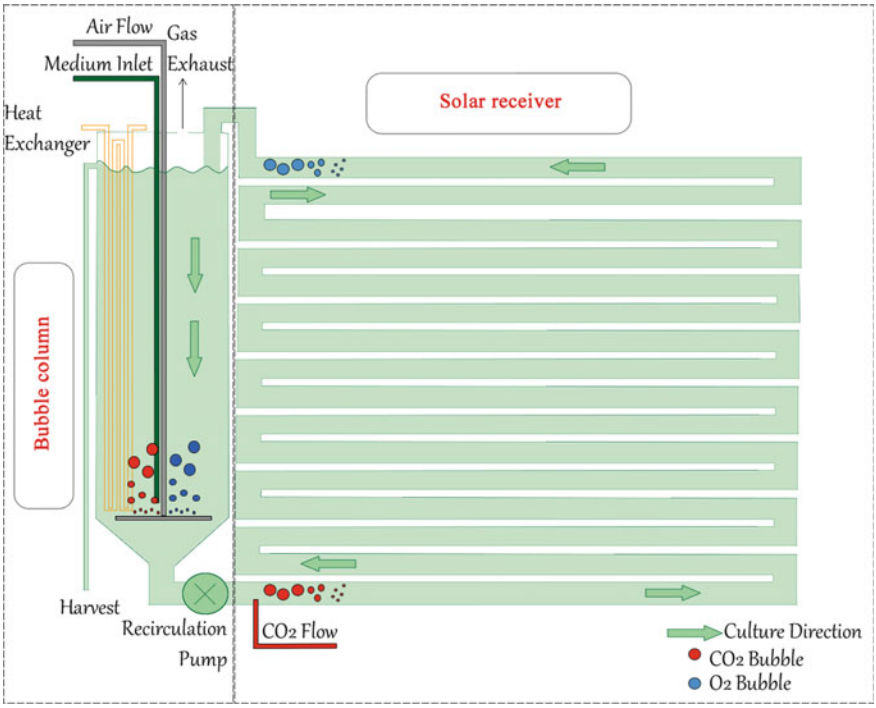

Fig. 2 Tubular photobioreactor scheme

ments, Spain), connected to a control transmitter unit MM44 (Crison Instrument, Spain); liquid and gas flow rates are measured using digital flow meters (PF2W540 and PF2A510, from SMC, Japan). All of these measures are in turn connected to a control computer through a data acquisition device NI Compact FieldPoint (National Instruments, USA). The complete system was designed and built by the Department of Chemical Engineering at the University of Almería (Spain), the control and data acquisition system was developed by the Department of Informatics at the University of Almería (Spain) using the development framework NI Labview (LabVIEW 2011 National Instrument, USA).

2.2 Raceway Photobioreactor

2.2.1 Microorganism and Culture Medium

As for the tubular photobioreactor, the microalgae strain used was *Scenedesmus almeriensis* (CCAP 276/24). However, in this case, experiments were performed using Arnon medium prepared with fertilizers instead of pure chemicals.

Fig. 3 Real view of the raceway reactor at the experimental station

2.2.2 Raceway Reactor and Operation Conditions

The raceway reactor used is located at 36° 48'N–2°43' W and also in Research Center "Las Palmerillas", property of Cajamar Foundation (Almería, Spain). The reactor consisted of two 50-m-length channels (0.46 m high × 1 m wide), both of them connected by 180° bends at each end, with a 0.59 m3 sump (0.65 m long × 0.90 m wide × 1 m deep) located 1 m part of the way down one channel (see Figs. 3 and 4). The entire reactor, including the sump, was made of white 3-mm-thick fiberglass. The liquid was circulated by a marine plywood paddle wheel with eight paddles, with a 1.2 m diameter, which is driven by an electric motor (Ebarba, Barcelona, Spain) with gear reduction and speed control using a frequency inverter (Ibérica, S.A. Barcelona, Spain). The reactor can be divided into three main parts depending on its fluid dynamic characteristics (channels, paddle wheel, and sump), such as observed in Fig. 4. For this reason, three pH-T and dissolved oxygen probes were situated at the end of each of these parts (5083T and 5120, Crison, Barcelona, Spain), connected to transmitters (MM44, Crison, Barcelona, Spain) and data acquisition software (Labview, National Instruments, USA). Air or CO_2 gas was automatically injected at the bottom of the sump through a diffuser to control the dissolved oxygen and pH of the culture. The gas flow rate entering to the reactor was measured by a mass flow meter (PFM 725S-F01-F, SMC, Tokyo, Japan).

Experiments were performed in semicontinuous mode. For this purpose, the reactor was filled with Arnon medium up to 15 cm water depth (15 m^3 volume), prepared from fertilizers instead of pure chemicals, and it was inoculated with a 10% total volume of culture from a 3.0 m^3 tubular photobioreactor. Then, it was operated in batch mode for 1 week. After that, the reactor was operated in semicontinuous mode at

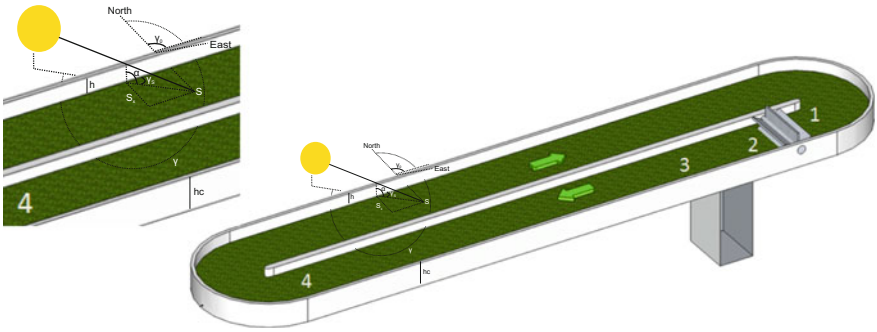

Fig. 4 Raceway reactor scheme showing dimensions. Numbers indicate the position where the probes were situated. (1) before paddle wheel, (2) after paddle wheel—before sump, (3) after sump—beginning of the channel, (4) end of the right channel

0.2 day^{-1}, this being previously demonstrated as optimal for this reactor (Mendoza et al. 2013b). To operate in semicontinuous mode, a fixed culture volume of 3.0 m^3 was harvested and replaced daily with the fresh medium over 6 h in the middle of the daylight period. Semicontinuous operation was maintained till steady state was achieved; only data around steady-state conditions being used. Evaporation (6–10 L/m^2 day^{-1}) inside the reactors was compensated by adding fresh medium, in addition to the volume of fresh medium used for the reactor' semicontinuous operation. The culture medium was not sterilized, simply filtered before entering the reactors using 200 μm pore-size filters to remove solids.

3 Dynamic Models

This section presents the nonlinear dynamic models for both tubular and raceway reactors. These models combine the fluid dynamic and mass transfer capacity of the reactors with the biological performance of the cells under different conditions. The models are based on mass balances, transport phenomena, thermodynamic relationships, and biological phenomena taking place into the system. Both models have been developed following the same ideas, since most of the physical–chemical balances are very similar in tubular and raceway reactors. So, temporal and spatial behaviors of the main variables of the system (such as biomass concentration, pH, dissolved oxygen, and total inorganic carbon in the liquid phase, in addition to oxygen and carbon dioxide exchange for the gas phase) are derived from the corresponding equations.

3.1 Modeling Issues

Microalgae cultures are composed of liquids, gases, and single-cell phototrophic microorganisms (considered as part of the liquid fraction of the system), whose productivity depends on the culture conditions to which the cells are exposed. Therefore, the first principle-based model must represent the physicochemical and biological phenomena that take place in the system, taking into account the relationships between light availability, culture conditions, and photosynthesis rate, besides the mixing and gas–liquid mass transfer inside the system. In outdoor cultures, the solar irradiance and temperature available depend on the location of the photobioreactor, while the rest of nutrients needed for the cells depend on design and operating conditions of the photobioreactor. Thereby, a general growth model for microalgal production system can be developed irrespective of photobioreactor type. Growth can be modeled by a function of the photosynthesis rate. The main parameter that determines the photosynthesis rate is the available light, based on external irradiance, culture characteristics, and reactor geometry (Acién et al. 1999, 2013). Thus, this fact will be first analyzed for each type of reactor to be related with the photosynthesis rate. Afterward, mass balances in liquid and gas states will be presented proving both spatial and temporal gradients for the main culture variables (Fernández et al. 2014, 2016).

3.2 Model for Tubular Photobioreactor

For the tubular reactor, the available light is calculated as a function of the total incident radiation on the photobioreactor surface, the light attenuation by biomass (Beer-Lambert law), and integrating local values over the total culture volume (Molina et al. 1996). However, bearing in mind a specific geometry and photobioreactor, this function can be simplified by Eq. (1) (Acién et al. 1997; Molina et al. 1996):

$$I_{av}(t,x) = \frac{I_0(t)\alpha_t}{K_{a,t}C_b(t,x)d_{t,p}}(1 - exp(-K_{a,t}C_b(t,x)d_{t,p})), \tag{1}$$

where t is the time, x is the space, I_0 is the solar irradiance on an obstacle-free horizontal surface, $K_{a,t}$ is the extinction coefficient, C_b is the biomass concentration, and $d_{t,p}$ is the tube diameter in the p part (where p can be substituted by l for the loop—solar receiver—and c for the bubble column). The solar irradiance has been modulated by a distribution factor α_t, which represents the solar irradiance fraction available in the particular area of the reactor.

The available average irradiance is correlated with the photosynthesis rate by a hyperbolical function as proposed in Costache et al. (2013), Molina et al. (1996a, b). This function is completed in this work by adding the rest of factors that limit the microalgal growth (under sufficient conditions of nutrients). So, the influences of the

pH culture value and dissolved oxygen of the culture have been modeled as described in Costache et al. (2013). Thus, a potential equation describes the influence of dissolved oxygen concentration on the photosynthesis rate, whereas for the temperature and pH conditions two models based on the Arrhenius equation were selected. The complete version for the photosynthesis rate is described by Eq. (2):

$$P_{O_{2,t}}(t,x) = \frac{P_{O_{2,max,t}} I_{av}(t,x)^{n_t}}{K_i exp(I_{av}(t,x)m_t) + I_{av}(t,x)^{n_t}} \left(1 - \left(\frac{[O_2](t,x)}{K_{O_2,t}}\right)^z\right) \left(B_1 exp\left(\frac{-C_1}{pH(t,x)}\right) - B_2 exp\left(\frac{-C_2}{pH(t,x)}\right)\right) - rP_{O_{2,max,t}}, \tag{2}$$

where $P_{O_{2,t}}$ is the photosynthesis rate (oxygen production rate per biomass mass unit), $P_{O_{2,max,t}}$ is the maximum photosynthesis rate for microorganisms under the culture conditions, $[O_2]$ is the dissolved oxygen concentration in liquid phase, n_t is the form exponent, and the term in the denominator is the irradiance constant, which increases as an exponential function of average irradiance, K_i and m_t being form parameters of this relationship, $K_{O_2,t}$ is the oxygen inhibition constant, and z is a form parameter. For the pH influence on the photosynthesis rate, B_1 and B_2 are the pre-exponential factors and C_1 and C_2 are the activation energies of the Arrhenius model. Furthermore, a factor r was included for the respiration phenomenon based on maximum photosynthesis rate.

On the other hand, the carbon dioxide uptake, $P_{CO_{2,t}}$, can be expressed as a one-to-one molar ratio between oxygen and carbon dioxide as follows:

$$P_{CO_{2,t}}(t,x) = -P_{O_{2,t}}(t,x). \tag{3}$$

While the biological phenomena are represented by the equations described above, the mixing, the gas–liquid mass transfer, and the heat transfer are explained in the next section. The balances, for the solar receiver, are formulated by means of several Partial Differential Equations (PDE) that lead to a distributed description of the process in the form of plug flow (approximation that allows to find a tradeoff between model performance and computational cost). On the other hand, the bubble column is considered as stirred tank perfectly mixing, being able to model it by Ordinary Differential Equations (ODE), although a plug flow approach can be also used.

3.2.1 Engineering Model of the Reactor

Tubular photobioreactors are composed of different parts: a solar receiver and a mixing unit, where the culture being recirculated from one to the other continuously using either airlift or mechanical pumps (see Fig. 2). The model of these processes must be applied to these different zones, since the mass transfer and fluid dynamics in each part are different, with variation for the position and time taking place in

each of them. Usually, the mixing unit is usually a bubble column, whereas the solar receiver is a continuous external tubular loop. In the bubble column, air is supplied for oxygen desorption, the liquid phase circulating through the column from the outlet to the inlet of the solar receiver. Thus, in this case, perfect mixing is considered to occur for both the liquid and gas phases. In the loop, the liquid is circulated by a centrifugal pump with pure CO_2 gas being supplied on demand for pH control. Therefore, plug flow has to be considered for the liquid and gas phases, and thus the external loop being divided into differential elements in which perfect mixing is assumed. The total number of differential elements is a function of the dispersion coefficient or mixing in the system (determined experimentally) (Fernández et al. 2012, 2014).

3.2.2 Mass Balances in the Liquid Phase

A mass balance for the biomass concentration can be defined as in Eq. (4), taking into account the photosynthesis process performed by the microalgae culture, and the transport phenomena due to the recirculation of the culture along the photobioreactor,

$$A_{liq,l}(t,x)\frac{\partial C_b(t,x)}{\partial t} = -Q_{liq,l}(t,x)\frac{\partial C_b(t,x)}{\partial x} + A_{liq,l}(t,x)P_{O_{2,t}}(t,x)C_b(t,x)Y_{o/x}, \tag{4}$$

where the subindex l refers to the solar receiver, $A_{liq,l}$ is the cross-sectional liquid area in the solar receiver that can be calculated as $A_{t,l}(1-\varepsilon_l(t,x))$, with $A_{t,l}$ being the total cross-sectional area of the loop and ε_l is the gas holdup, $Q_{liq,l}$ is the volumetric flow rate of liquid defined as $VA_{liq,l}$, where $V(t)$ is the velocity of the fluid established by the centrifugal pump of the photobioreactor, and $Y_{o/x}$ is the biomass yield coefficient produced by the oxygen unit mass.

In the bubble column, a similar balance can be considered by an ordinary differential equation where the spatial dimension is removed (although as has been pointed before, plug flow could also be considered). Furthermore, since the dilution process is performed in this part of the photobioreactor, an output biomass concentration has been added driven by the volumetric flow rate of medium, Eq. (5).

$$V_{liq,c}(t)\frac{dC_{b,out}(t)}{dt} = -Q_{liq,c}(t)(C_{b,out}(t) - C_{b,in}(t)) + V_{liq,c}(t)P_{O_{2,t}}(t)C_{b,out}(t)Y_{o/x} - Q_m(t)C_{b,out}(t), \tag{5}$$

where the subindex c refers to the bubble column, $V_{liq,c}$ is the liquid volume, which can be calculated as $V_{t,c}(1-\varepsilon_c(t))$ where $V_{t,c}$ is the total volume and ε_c is the gas holdup, $Q_{liq,c}$ is the volumetric flow rate of liquid, $C_{b,out}$ is the outlet biomass concentration (solar receiver input), $C_{b,in}$ is the inlet biomass concentration (solar receiver output), and Q_m is the volumetric flow rate of culture medium.

Regarding dissolved oxygen concentration, it can be related to the gas–liquid mass transfer rate and the photosynthesis rate by the following mass balance:

$$A_{liq,l}(t,x)\frac{\partial[O_2](t,x)}{\partial t} = -Q_{liq,l}(t,x)\frac{\partial[O_2](t,x)}{\partial x} + A_{liq,l}(t,x)\frac{P_{O_{2,t}}(t,x)C_b(t,x)}{M_{O_2}} + A_{liq,l}(t,x)K_l a_{l,O_2 l}(t,x)([O_2^*](t,x) - [O_2](t,x)), \tag{6}$$

where $[O_2]$ is the dissolved oxygen concentration in liquid phase, M_{O_2} is the molecular weight of oxygen, $K_l a_{l,O_2 l}$ is the volumetric gas–liquid mass transfer coefficient for oxygen, and $([O_2^*] - [O_2])$ is the mean driving force. The equilibrium concentration in gas phase $[O_2^*]$ is calculated as a function of the oxygen concentration in the gas phase based on Henry's law by Eq. (7):

$$[O_2^*](t,x) = H_{O_2} P_T y_{O_2}(t,x), \tag{7}$$

where H_{O_2} is the Henry's constant for oxygen, P_T is the total pressure, and y_{O_2} is the oxygen molar fraction in the gas phase.

The homologous balance for the bubble column must consider the dissolved oxygen concentration in the input medium liquid. Thus, the next balance can be established as

$$V_{liq,c}(t)\frac{d[O_2]_{out}(t)}{dt} = -Q_{liq,c}(t)([O_2]_{out}(t) - [O_2]_{in}(t)) + V_{liq,c}(t)\frac{P_{O_{2,t}}(t)C_{b,out}(t)}{M_{O_2}} + V_{liq,c}(t)K_l a_{l,O_2 c}(t)([O_2^*](t) - [O_2](t))_{ml} - Q_m(t)([O_2]_{m,t} - [O_2]_{out}(t)), \tag{8}$$

where $[O_2]_{in}$ and $[O_2]_{out}$ are the oxygen concentrations in liquid phase at the inlet and outlet of the bubble column, $K_l a_{l,O_2 c}$ is the volumetric gas–liquid mass transfer coefficient for oxygen in the bubble column, $([O_2^*] - [O_2])_{ml}$ is a logarithmic mean driving force, and $[O_2]_{m,t}$ is the dissolved oxygen in the culture medium.

Regarding inorganic total carbon concentration, it can be calculated by a mass balance to the liquid phase in a similar way to dissolved oxygen by Eq. (9):

$$A_{liq,l}(t,x)\frac{\partial[C_T](t,x)}{\partial t} = -Q_{liq,l}(t,x)\frac{\partial[C_T](t,x)}{\partial x} + A_{liq,l}(t,x)\frac{PCO_{2,t}(t,x)C_b(t,x)}{M_{CO_2}} + A_{liq,l}(t,x)K_l a_{l,CO_2 l}(t,x)([CO_2^*](t,x) - [CO_2](t,x)), \tag{9}$$

where $K_l a_{l,CO_2 l}$ is the mass transfer coefficient for CO_2, and total inorganic carbon in the liquid phase is defined as $[C_T]$, which depends on the carbon dioxide concentration in the liquid phase $[CO_2]$ and the equilibrium concentration in the gas phase $[CO_2^*]$. The equilibrium concentration can be calculated, according to Henry's law, as a function of Henry's constant, H_{CO_2}, the total pressure P_T and the molar fraction of CO_2 in the gas phase, y_{CO_2}.

For the bubble column, the inorganic carbon concentration from culture medium must be regarded in the balance as shown in Eq. (10):

$$\begin{aligned} &V_{liq,c}(t)\frac{d[C_T]_{out}(t)}{dt} = -Q_{liq,c}(t)([C_T]_{out}(t) - [C_T]_{in}(t)) + \\ &V_{liq,c}(t)\frac{P_{CO_{2,t}}(t)C_{b,out}(t)}{M_{CO_2}} + V_{liq,c}(t)K_l a_{l,CO_2 c}(t)([CO_2^*](t) - [CO_2](t))_{lm} - \\ &Q_m(t)([C_T]_{m,t} - [C_T]_{out}(t)), \end{aligned} \tag{10}$$

where $K_l a_{l,CO_2 c}$ is the mass transfer coefficient for CO_2 in the bubble column. The total inorganic carbon is defined at the inlet $[C_T]_{in}$ and outlet $[C_T]_{out}$ of the bubble column, and $[C_T]_{m,t}$ is the inorganic carbon concentration in the culture medium.

The pH value is defined as the decimal logarithm of the hydrogen concentration in the system, $-log10([H^+])$. Several equilibrium relations can be found between the hydrogen concentration and carbon species in the system (dissolved carbon dioxide, carbonate, $[HCO_3^-]$, and bicarbonate, $[CO_3^{2-}]$) as can be seen in Fernández et al. (2012).

3.2.3 Mass Balances in the Gas Phase

In addition to the liquid phase, CO_2 injections in gaseous form are incorporated in order to adjust the pH and neutralize the carbon lack in the system during photosynthesis process. On the other hand, air injections are demanded in the bubble column to control high levels of dissolved oxygen accumulated into the loop. Therefore, mass balances on the gas phases are needed to include these phenomena. Since the nitrogen molar fraction can be considered constant because its solubility is approximately zero, the balances presented here are formulated by relations from the rest of gases to nitrogen molar ratio. Regarding the oxygen, the next balance, Eq. (11), can be established:

$$\begin{aligned} &A_{gas,l}(t,x)\frac{\partial Y_{O_2}(t,x)}{\partial t} = -\frac{F_{N_{2,l}}(t,x)V_{mol}}{y_{N_{2,l}}}\frac{\partial Y_{O_2}(t,x)}{\partial x} - \\ &\frac{A_{liq,l}(t,x)V_{mol}}{y_{N_{2,l}}}K_l a_{l,O_2 l}(t,x)([O_2^*](t,x) - [O_2](t,x)), \end{aligned} \tag{11}$$

where $A_{gas,l}$ is the cross-sectional gas area, which can be calculated as $A_{t,l}\varepsilon_l(t,x)$, V_{mol} is the molar volume under reactor conditions (pressure and temperature), Y_{O_2} is the oxygen-to-nitrogen molar ratio in the gas phase, $F_{N_{2,l}}$ is the molar flow rate of nitrogen in the gas phase, and $y_{N_{2,l}}$ is the nitrogen molar fraction used in the solar receiver. For the column, a similar mass balance can be considered taking into account the gas characteristics injected in this section. Thus, an ODE can be written as shown in Eq. (12):

$$V_{gas,c}(t)\frac{dY_{O_2,out}(t)}{dt} = -\frac{F_{N_{2,c}}(t)V_{mol}}{y_{N_{2,c}}}(Y_{O_2,out}(t) - Y_{O_2,in}(t)) - \frac{V_{liq,c}(t)V_{mol}}{y_{N_{2,c}}}K_l a_{l,O_2c}(t)([O_2^*](t) - [O_2](t))_{lm}, \tag{12}$$

where the oxygen-to-nitrogen molar ratio in the gas phase is defined at the inlet $Y_{O_2,in}$ and outlet $Y_{O_2,out}$ of bubble column, $V_{gas,c}$ is the gas volume, which can be calculated as $V_{t,c}\varepsilon_c(t)$, $F_{N_{2,c}}$ is the molar flow rate of nitrogen for the bubble column, and $y_{N_{2,c}}$ is the nitrogen molar fraction used in the bubble column. For the carbon dioxide, an analogous mass balance can be defined by Eq. (13):

$$A_{gas,l}(t,x)\frac{\partial Y_{CO_2}(t,x)}{\partial t} = -\frac{F_{N_{2,l}}(t,x)V_{mol}}{y_{N_{2,l}}}\frac{\partial Y_{CO_2}(t,x)}{\partial x} - \frac{A_{liq,l}(t,x)V_{mol}}{y_{N_{2,l}}}K_l a_{l,CO_2l}(t,x)([CO_2^*](t,x) - [CO_2](t,x)), \tag{13}$$

where Y_{CO_2} is the carbon dioxide to nitrogen molar ratio in the gas phase, $F_{N_{2,l}}$ is the molar flow rate of nitrogen in the gas phase, and $y_{N_{2,l}}$ is the nitrogen molar fraction used in the solar receiver. The perfectly mixing version for the bubble column is represented by Eq. (14):

$$V_{gas,c}(t)\frac{dY_{CO_2,out}(t)}{dt} = -\frac{F_{N_{2,c}}(t)V_{mol}}{y_{N_{2,c}}}(Y_{CO_2,out}(t) - Y_{CO_2,in}(t)) - \frac{V_{liq,c}(t)V_{mol}}{y_{N_{2,c}}}K_l a_{l,CO_2c}(t)([CO_2^*](t) - [CO_2](t))_{lm}, \tag{14}$$

where the carbon dioxide to nitrogen molar ratio in the gas phase is defined at the inlet $Y_{CO_2,in}$ and outlet $Y_{CO_2,out}$ of bubble column.

In both mass balances, molar ratio to nitrogen is used instead of molar fraction. However, a relationship between these units is known by Eq. (15):

$$y = \frac{Y}{1+Y}. \tag{15}$$

An improvement has been developed taking into account the nitrogen gas transport since, although this element can be constant due to lack of mass transfer, a transport effect is produced when a gas bubble is injected in the loop up to finally leaves it. Assuming the same velocity for each component of the gas flow rate and no slip between the liquid phase and the gas phase, the gas transport can be modeled by changes in the cross-sectional area of the nitrogen A_{N_2} along the tube, being able to describe these changes by the following balance:

$$A_{gas,l}(t,x)\frac{\partial A_{N_{2,l}}(t,x)}{\partial t} = -\frac{F_{N_{2,l}}(t,x)V_{mol}}{y_{N_{2,l}}}\frac{\partial A_{N_{2,l}}(t,x)}{\partial x}, \tag{16}$$

where $A_{N_{2,l}}$ is the cross-sectional area of the nitrogen in the solar receiver.

A relation can be found between the molar flow rate of nitrogen and the gas flow rate along the tube by Eq. (17):

$$F_{N_{2,l}}(t,x) = \frac{Q_{gas,l}(t,x)y_{N_{2,l}}}{V_{mol}}, \tag{17}$$

where the volumetric flow rate of gas $Q_{gas,l}$ can be established as the sum of the three volumetric flow rates which take place into the loop (carbon dioxide, oxygen, and nitrogen). Therefore, a relationship between the volumetric flow rate of gas and the cross-sectional nitrogen area can be calculated using the molar ratio to nitrogen for the rest of components as

$$Q_{gas,l}(t,x) = VA_{N_{2,l}}(t,x)(1 + Y_{O_2}(t,x) + Y_{CO_2}(t,x)). \tag{18}$$

On the other hand, the gas holdup determines the mass transfer in both the bubble column and the solar receiver. Bearing in mind physical characteristics of each part of the system, different models of the gas holdup were modeled. For the solar receiver, assuming no slip between the liquid phase and the gas phase, the gas holdup expression can be approximated by Eq. (19):

$$\varepsilon_l(t,x) = \frac{Q_{gas,l}(t,x)}{Q_{gas,l}(t,x) + Q_{liq,l}(t,x)}. \tag{19}$$

In the bubble column, a slip velocity exits between the gas and the liquid phases. Therefore, a drift flux model can be used to predict the gas holdup (Zuber and Findlay 1965), which is given by Eq. (20):

$$\varepsilon_c(t) = \frac{U_{gas}(t)}{(C_o U_{gas}(t) + U_{liq}(t)) + U_\infty}, \tag{20}$$

where U_{gas} and U_{liq} are the superficial velocity of the gas and liquid, respectively. C_o is a drift flux model parameter and U_∞ is the bubble accession rate.

Therefore, the mass transfer coefficient can be defined as a function of the gas holdup according to the part of the system that is modeled (Chisti and Moo-Young 1987). Even further, the mass transfer coefficient for the CO_2 is directly related to the mass transfer coefficient for the oxygen by the difference in aqueous diffusivity of the two gases (K_{CO_2}) as follows (Molina et al. 1993), Eqs. (21) and (22):

$$K_l a_{l,O_2 l}(t,x) = a_l \varepsilon_l(t,x)^{b_l} \qquad K_l a_{l,CO_2 l}(t,x) = K_{CO_{2,l}} K_l a_{l,O_2 l}(t,x) \tag{21}$$

$$K_l a_{l,O_2 c}(t) = a_c \varepsilon_c(t)^{b_c} \qquad K_l a_{l,CO_2 c}(t) = K_{CO_{2,c}} K_l a_{l,O_2 c}(t). \tag{22}$$

$K_{CO_{2,l}}$ and $K_{CO_{2,c}}$ are the transfer coefficient constants for CO_2 at the solar receiver and at the bubble column, respectively; whereas a_l, b_l and a_c, b_c are form parameters adjusted to each part of the photobioreactor.

Another possible characterization of the mass transfer coefficients can be given by relating the volumetric interfacial area, Eq. (23), between the gas and the liquid phases:

$$K_l a_{l,O_2}(t,x) = K_l a_i(t,x), \tag{23}$$

where K_l is the liquid-side mass transfer coefficient, and a_i is the interfacial area which can be calculated by the initial bubble diameter, d_b, and the gas holdup in each loop section as

$$a_i(t,x) = \frac{6\varepsilon_l(t,x)}{d_b(1-\varepsilon_l(t,x))}. \tag{24}$$

3.3 Model for Raceway Photobioreactor

Such as mentioned above, the solar radiation availability is the first element to be analyzed when a model of this type is developed. For the raceway reactor (as horizontal surface), light availability can be easily estimated using classical solar radiation equations. However, the net amount of light received in raceway reactors is a function of its design, especially the walls shadow having a large influence. In this sense, shadow is generated by the channel walls in each cross-sectional area of the reactor, depending on the sun position and reactor geometry. Therefore, shadow influences the photosynthesis rate, and it can be modeled as a distributed factor (α_s) in each cross-sectional area. The shadow factor (α_s) is calculated taking into account the length of the shadow projection on the perpendicular axis of the walls, such as shown in Fig. 4, using Eqs. (25) and (26) (Kittler and Darula 2013). According to these equations, azimuth (α) and altitude angles (γ) are calculated as a function of the latitude (ϕ), hour angle (ω), and sun declination (δ), these last two terms being a function of the day of the year (N) and the solar hour (h_s). The projection of the shadow generated by the channel walls onto the surface of the cross-sectional area (s_x) can be described in terms of the wall height (h_w), the solar altitude angle, the Azimuth angle, and the angle measured from the North to the normal vector of the cross-sectional area of the reactor (γ_0), in this case 84° (Fig. 4). Finally, the distributed parameter is calculated as the ratio to the total width of the channel as follows:

$$\alpha = \sin^{-1}(\sin(\delta)\sin(\phi) + \cos(\delta)\cos(\phi)\cos(\omega)) \tag{25}$$

$$\gamma = \cos^{-1}\left(\frac{\sin(\delta)\cos(\phi) - \cos(\delta)\cos(\omega)\sin(\phi)}{\cos(\alpha)}\right) \tag{26}$$

$$\begin{aligned} &\text{if } \sin(\omega) > 0 \text{ then } \gamma = 360 - \gamma \\ &\text{if } \sin(\omega) \leq 0 \text{ then } \gamma = \gamma \end{aligned} \tag{27}$$

$$\delta = 23.45 \sin\left(\frac{360(284 + N)}{365}\right) \tag{28}$$

$$\omega = 15(12 - h_s) \tag{29}$$

$$s_x = \text{abs}\left(\frac{(h_w - h) * \sin(\gamma - (180 + \gamma_0))}{tan(\alpha)}\right) \tag{30}$$

$$\alpha_s = \frac{s_x}{w}. \tag{31}$$

Once the distributed factor, α_s, is estimated, the average irradiance (I_{av}) can be obtained. The average irradiance integrates the local irradiance values inside the culture over the total culture volume, being calculated as a function of the total incident radiation on the photobioreactor surface (I_o), the biomass concentration (C_b), the light attenuation of the biomass ($K_{a,r}$), and the light path or culture depth (h) (Molina et al. 1996a). Taking into account the variation of biomass concentration with time, t, and position along the reactor, x, the average irradiance in whatever section of the reactor can be calculated by Eq. (32):

$$I_{av}(t,x) = \frac{I_0(t)}{K_{a,r}C_b(t,x)h}(1 - exp(-K_{a,r}C_b(t,x)h). \tag{32}$$

The photosynthesis rate ($P_{O_{2,r}}$), defined as the oxygen production rate per biomass mass unit, is correlated with the average irradiance by an hyperbolic function, the response of photosynthesis rate to average irradiance being modulated by adequacy of culture conditions using normalized factors (Costache et al. 2013). A potential equation describes the influence of dissolved oxygen concentration on the photosynthesis rate, whereas for the pH a model based on the Arrhenius equation is used. Thus, under nutrient-sufficient conditions, equation (33) can be used to determine the photosynthesis rate as a function of average irradiance, dissolved oxygen concentration ($[O_2]$), and pH into the culture (Costache et al. 2013). In this equation, several biological parameters specific of microalgae strain and growth status of the cells are included, as the maximum photosynthesis rate under the culture conditions ($P_{O_{2,\max,r}}$), the form exponent (n_r), the irradiance constant (as an exponential function of average irradiance, K_i and m_r), the oxygen inhibition constant ($K_{O_{2,r}}$), a form parameter (z), the pre-exponential factors (B_1, B_2), the activation energies of the Arrhenius model (C_1, C_2), and the constant respiration rate (R_{O_2}):

$$P_{O_{2,r}}(t,x) = (1-\alpha_s)\frac{P_{O_{2,max,r}} I_{av}(t,x)^{n_r}}{K_i exp(I_{av}(t,x)m_r) + I_{av}(t,x)^{n_r}} \left(1 - \left(\frac{[O_2](t,x)}{K_{O_{2,r}}}\right)^z\right) \left(B_1 exp\left(\frac{-C_1}{pH(t,x)}\right) - B_2 exp\left(\frac{-C_2}{pH(t,x)}\right)\right) - \alpha_s R_{O_2}. \quad (33)$$

Once the photosynthesis rate is modeled, Eq. (34) allows determining the biological carbon dioxide uptake ($P_{CO_{2,r}}$) considering a one-to-one molar ratio between oxygen and carbon dioxide (from basic equation of photosynthesis). Moreover, considering a mean value of oxygen coefficient yield ($Y_{b/O2}$), the net production of biomass can be determined by Eq. (35):

$$P_{CO_{2,r}}(t,x) = -P_{O_{2,r}}(t,x) \quad (34)$$

$$P_b(t,x) = Y_{b/O_2}(t,x) P_{O_{2,r}}(t,x). \quad (35)$$

3.3.1 Engineering Model of the Reactor

The raceway reactor used in this work has been previously characterized in both fluid dynamic and mass transfer capacity (Godos et al. 2014; Mendoza et al. 2013a, b). According to this previous knowledge, the reactor can be divided into three main zones: channel, paddle wheel, and sump. The channel performs as a plug flow reactor, thus perfect mixing exits into the cross section of the channel, and axial gradients are considered due to biological and mass transfer phenomena. Biological phenomena (production of oxygen, consumption of inorganic carbon, production of biomass, etc.) take place into the channel, in addition to mass transfer between the culture and the atmosphere (oxygen and carbon dioxide exchange). Paddle wheel performs as stirred tank, thus perfect mixing existing in the liquid phase with no gradients taking place. In this section of the reactor, biological phenomena also take place in addition to mass transfer between liquid and atmosphere. The sump performs also as stirred tank for the liquid, and thus no gradients exist. However, as plug flow for the injected gas is considered, gradients of oxygen and carbon dioxide into the gas phase appear. Inside the sump, the same biological phenomena take place, but the mass transfer is a function of gas phase composition along the sump. Assuming constant velocity (V) and liquid height (h) inside the channel, the volumetric flow rate of liquid (Q_{liq}) is defined as the multiplication of velocity and cross-sectional area of the channel (calculated using the liquid height and the width of the channel, w). This flow rate is constant for the three sections of the reactor. Regarding the mass transfer, it is a function of mass transfer coefficient and driving force in each position, the driving force being a function of the component concentration into the liquid phase and that in equilibrium with the gas phase in contact.

To model the raceway reactor, mass balances have been applied to each reactor section. A model using partial differential equations (PDEs) has been used to cope

with the existence of plug flow behavior in some parts of the reactor. PDEs are used in many physical problems, such as fluid flow, heat transfer, solid mechanics, and biological processes. Only ordinary differential equation (ODE) has been applied to stirred tank sections of the reactor as sump and paddle to reduce the computational effort (Fernández et al. 2016).

3.3.2 Mass Balances in the Liquid Phase

The three main components considered into the liquid phase are biomass concentration (C_b), dissolved oxygen concentration ($[O_2]$), and total inorganic carbon concentration ($[C_T]$). A mass balance is defined for each one of the three components in each section of the reactor. Thus, the proposed balances for each one of the three components are shown in equations (36)–(38):

$$wh\frac{\partial C_b(t,x)}{\partial t} = -whV_r\frac{\partial C_b(t,x)}{\partial x} + whP_{O_2}(t,x)C_b(t,x)Y_{b/O_2} \tag{36}$$

$$\begin{aligned} wh\frac{\partial [O_2](t,x)}{\partial t} &= -whV_r\frac{\partial [O_2](t,x)}{\partial x} + \\ &+ wh\frac{P_{O_{2,r}}(t,x)C_b(t,x)}{M_{O_2}} + whK_{laO_{2_{ch}}}([O_2^*](t,x) - [O_2](t,x)) \end{aligned} \tag{37}$$

$$\begin{aligned} wh\frac{\partial [C_T](t,x)}{\partial t} &= -whV\frac{\partial [C_T](t,x)}{\partial x} + \\ &+ wh\frac{P_{CO_{2,r}}(t,x)C_b(t,x)}{M_{CO_2}} + whK_{laCO_{2_{ch}}}([CO_2^*](t,x) - [CO_2](t,x)). \end{aligned} \tag{38}$$

The oxygen mass transfer is a function of the volumetric coefficient for oxygen into the channel ($K_{laO_{2_{ch}}}$) and the logarithmic driving force ($[O_2^*]-[O_2]$) (Camacho et al. 1999). The dissolved oxygen concentration in equilibrium with air surrounding the channel ($[O_2^*]$) is calculated as a function of the oxygen molar fraction into the air (0.21) based on Henry's law. The carbon dioxide mass transfer is calculated in the same way as a function of volumetric mass transfer for carbon dioxide into the channel ($K_{laCO_{2_{ch}}}$), which could be directly related to $K_{laO_{2_{ch}}}$ by a factor of 0.93, which takes into account the difference in aqueous diffusivity of the two gases. Regarding the carbon dioxide concentration ($[CO_2]$), it is a function of total inorganic carbon ($[C_T]$) and pH, due to the existence of bicarbonate buffer (Camacho et al. 1999). The carbon dioxide concentration in equilibrium with the gas phase ($[CO_2^*]$) is also calculated as a function of the carbon dioxide molar fraction into the air (0.0003) based on Henry's law.

Analogous mass balances are applied to the paddle wheel, considering that this section can be represented by ODEs. For the paddle wheel, the concentration of

the major components at inlet is that calculated as outlet from the channel. Equations (39)–(41) allow us calculating the biomass, dissolved oxygen, and inorganic carbon concentration at the outlet of the paddle wheel taking into account the specific dimensions and mass transfer coefficients in this section. It is important to note that in paddle wheel, air is also the gas in contact with the liquid phase, its concentration being constant in spite of oxygen and carbon dioxide exchange:

$$\frac{dC_{b,out}(t)}{dt} = -\frac{Q_{liq}}{V_p}(C_{b,out}(t) - C_{b,in}(t)) + \\ + P_{O_{2,r}}(t)C_{b,in}(t)Y_{b/O_2} \tag{39}$$

$$\frac{d[O_2]_{out}(t)}{dt} = -\frac{Q_{liq}}{V_p}([O_2]_{out}(t) - [O_2]_{in}(t)) + \\ + \frac{P_{O_{2,r}}(t)C_{b,out}(t)}{M_{O2}} + K_{laO_{2_p}}([O_2^*](t) - [O_2](t))_{lm} \tag{40}$$

$$\frac{d[C_T]_{out}(t)}{dt} = -\frac{Q_{liq}}{V_p}([C_T]_{out}(t) - [C_T]_{in}(t)) + \\ + \frac{P_{CO_{2,r}}(t)C_{b,out}(t)}{M_{CO2}} + K_{laCO_{2_p}}([CO_2^*](t) - [CO_2](t))_{lm}. \tag{41}$$

Similar mass balances are applied to the sump also considering that this section can be represented by ODEs. For the sump, the concentration of major components at inlet is that calculated as outlet from the paddle wheel. Equations (42)–(44) allow us calculating the biomass, dissolved oxygen, and inorganic carbon concentration at the outlet of the sump, respectively:

$$\frac{dC_{b,out}(t)}{dt} = -\frac{Q_{liq}}{V_s(1-\varepsilon_s(t))}(C_{b,out}(t) - C_{b,in}(t)) + \\ + P_{O_{2,r}}(t)C_{b,in}(t)Y_{b/O_2} - \frac{Q_m}{V_s(1-\varepsilon_s(t))}C_{b,out}(t) \tag{42}$$

$$\frac{d[O_2]_{out}(t)}{dt} = -\frac{Q_{liq}}{V_s(1-\varepsilon_s(t))}([O_2]_{out}(t) - [O_2]_{in}(t)) + \\ + \frac{P_{O_{2,r}}(t)Cb_{out}(t)}{M_{O2}} + K_{laO_{2_s}}([O_2^*](t) - [O_2](t))_{lm} + \\ + \frac{Q_m}{V_s(1-\varepsilon_s(t))}([O_2]_m - [O_2]_{out}(t)) \tag{43}$$

$$\frac{d[C_T]_{out}(t)}{dt} = -\frac{Q_{liq}}{V_s(1-\varepsilon_s(t))}([C_T]_{out}(t) - [C_T]_{in}(t)) + \\ + \frac{P_{CO_{2,r}}(t)Cb_{out}(t)}{M_{CO2}} + K_{laCO_{2_s}}([CO_2^*](t) - [CO_2](t))_{lm} + \\ + \frac{Q_m}{V_s(1-\varepsilon_s(t))}([C_T]_{m,r} - [C_T]_{out}(t)). \tag{44}$$

Since air or carbon dioxide is injected into the sump, and the exchange of oxygen and carbon dioxide from the gas phase to the liquid modifies the oxygen and carbon dioxide molar fraction into the gas phase, the oxygen and carbon dioxide in the liquid equilibrium with the gas phase must be dynamically calculated. The outlet of biomass, dissolved oxygen, and inorganic carbon from the system due to harvesting is included by considering the volumetric flow of medium (Q_m). In these equations, the volume of each section is corrected by gas holdup (ε_s) to determine the right liquid volume in each section. The gas holdup can be approximated by Eq. (45) taking into account the difference between the volumetric flow rate of gas introduced in the sump, Q_{gas}, and the total volumetric flow rate of the system (Q_{gas}+Q_{liq}):

$$\varepsilon_s(t) = \frac{Q_{gas}(t)}{Q_{gas}(t) + Q_{liq}(t)}. \tag{45}$$

To apply these equations, the different volumes of each section are considered, and thus the volume of the sump is calculated by Eq. (46) considering the height (h_{ss}), wide (w_s), and length (l_s) of the sump in addition to the volume of channel comprises over the sump:

$$V_s = h_{ss}w_s l_s + hw_s l_s. \tag{46}$$

3.3.3 Mass Balances to the Gas Phase

Air (21% O_2, 0.03% CO_2) or flue gas (6% O_2, 10% CO_2) is injected into the sump to control the dissolved oxygen concentration and the pH of the culture. Thus, when the pH is higher than set point (pH = 8), flue gas is injected to reduce the pH and supply inorganic carbon, otherwise air being injected to minimize the accumulation of dissolved oxygen and to avoid achieving toxic dissolved oxygen concentrations (>250% Sat) (Costache et al. 2013). The injected gas modifies its composition along the sump due to mass transfer, and thus mass balances are also applied to the gas phase to determine its variation along the sump. Since the nitrogen molar flow can be considered constant, because its solubility is low, the balances are formulated by relations from the rest of gases to nitrogen molar ratio. According to Eq. (47), the variation of the oxygen-to-nitrogen molar ratio into the gas phase (Y_{O_2}) is a function of the nitrogen molar fraction into the gas phase (y_{N_2}). For the carbon dioxide,

an analogous mass balance can be defined to determine the variation of the carbon dioxide to nitrogen molar ratio in the gas phase (Y_{CO_2}):

$$\begin{aligned}\frac{dY_{O_2,out}(t)}{dt} &= -\frac{Q_{gas}}{V_s(1-\varepsilon_s(t))}(Y_{O_2,out}(t) - Y_{O_2,in}(t)) \\ &- K_{laO_{2_s}}\frac{V_{mol}}{y_{N_2}}\frac{(1-\varepsilon_s(t))}{\varepsilon_s(t)}([O_2^*](t) - [O_2](t))_{lm}\end{aligned} \tag{47}$$

$$\begin{aligned}\frac{dY_{CO_2,out}(t)}{dt} &= -\frac{Q_{gas}}{V_s(1-\varepsilon_s(t))}(Y_{CO_2,out}(t) - Y_{CO_2,in}(t)) \\ &- K_{laCO_{2_s}}\frac{V_{mol}}{y_{N_2}}\frac{(1-\varepsilon_s(t))}{\varepsilon_s(t)}([CO_2^*](t) - [CO_2](t))_{lm}.\end{aligned} \tag{48}$$

3.4 Solvers and Software

PDEs and ODEs balances, which establish the base of the model, have been implemented by the software Matlab 8.3 (MathWorks, Massachusetts, USA). Since the computational cost required to solve these kinds of equations is high, a general procedure for the first-order hyperbolic equations has been used by means of the well-known method of lines for PDE equations. On the other hand, ODEs balances have been calculated by a forward first-order finite difference approximation method. Note that for the calibration procedure a multidimensional nonlinear minimization process has been formulated, using Sequential Quadratic Programming (SQP) methods since a Quadratic Programming (QP) subproblem was considered. For that reason, an appropriated simulation time of the model is required in order to solve the optimization problem suggested in a reasonable time. Model calibration has been performed comparing real data of pH and dissolved oxygen concentration at the end of the different sections of the reactors, with the simulation response obtained using estimated values of characteristic parameters.

4 Results

This section summarizes the main results obtained for both models. First, calibration and validation results are presented using experimental data, and afterward different analyses are derived by using the resulting models as design/analysis tools (Fernández et al. 2012, 2014, 2016).

The calibration and validation of a biological system is a very complex task due to the large number of experimental tests that must be performed and the number of parameters that have to be calibrated, many of them depending on the culture con-

ditions. For that reason, a suitable methodology is to divide these parameters into different groups depending on their characteristics, for example, biological and fluid dynamic parameters can be separated in order to perform specific tests for each one. Physical and chemical parameters, such as mass and heat transfer coefficients, can be determined by experimental data without culture or using known fluid dynamic relationships (Acién et al. 2001; Camacho et al. 1999). On the other hand, biological parameters can be calculated at laboratory scale, where a lot of conditions can be evaluated, although they must be readjusted in outdoor culture conditions and other scales (Costache et al. 2013; Sánchez et al. 2008a, b). In addition, the whole system can be adjusted from experimental data of outdoor cultures, by fitting these data into the responses of the proposed model. Error metrics, such as integrated absolute error (IAE) or integrated squared error (ISE), can be used for this purpose, formulating an optimization problem for the calibration process. However, the use of experimental data of outdoor cultures must be treated carefully due to different reasons. First, noise and other disturbances must be filtered from the experimental data in order to remove possible uncertainties in the optimization problem. Second, the photosynthesis rate, biological part of the model, is an equation composed by different kinetic equations related to the culture conditions, and thus specific tests must be performed under controlled conditions for an appropriate calibration. However, this process is difficult to carry out in outdoor conditions, and therefore certain constraints and a suitable initial point, based on parameters obtained in laboratory scale, must be established in the calibration problem. Finally, possible disturbances must be considered, above all those affecting the pH variable. Since the pH value is very sensitive to changes in other variables as total inorganic carbon, some disturbances can appear due to inorganic carbon concentration added in the culture medium dilution during the operation in continuous mode, as well as small differences in the mass transfer coefficients because of biological reactions produced during the photosynthesis process.

4.1 Results for Tubular Photobioreactor

This section describes the results obtained for the tubular photobioreactor. From previous works (Camacho et al. 1999; Costache et al. 2013; Mendoza et al. 2013a) and significant knowledge of the processes, experimental data from outdoor culture were only needed to fit the model response from biological parameters obtained in laboratory scale and fluid dynamic parameters obtained from a similar photobioreactor structure. A wide range of solar radiation conditions was covered (around 2 months of data with sunny and cloudy days), where the culture was operated in continuous mode at 0.34 l day^{-1}. The volumetric flow rate of air was constant at 140 l min^{-1}, allowing to capture the kinetic properties of the photosynthesis rate through the dissolved oxygen variable. The available data have been divided into two sets, one for calibration and another for validation purposes. Regarding CO_2 injections, an analysis on the pH was carried out in order to set up profiles of input signals that allow

to regulate the pH, avoiding damages to the culture, and at the same time, capturing dynamics related to CO_2 injection. Multilevel PRBSs were performed by a pulse-width modulation due to the discontinuous nature of the CO_2 valve, and these signals were adapted according to the period of day to keep the pH value in an appropriate and secure range (Sánchez et al. 2008a, b). Several data were registered remaining the volumetric flow rate around 30 l min^{-1}. Finally, the velocity of the culture flow was fixed at 1 m s^{-1} (notice that working with constant velocity is the typical way of operating these kinds of systems, although the developed model can cope with changing conditions on this variable).

4.1.1 Model Calibration and Validation

Regarding calibration and validation of the model, experimental data of solar radiation, biomass concentration, pH, and dissolved oxygen were required for calibration and validation steps. These problems were divided into two periods, night period when microalgae build up CO_2 due to respiration process, and light period when the photosynthesis rate is produced and CO_2 is consumed. Mass transfer parameters for the loop section (a_l and b_l, Eq. (21)) were calibrated with pH values (due to its higher influence on the CO_2 injections) during night periods, where the solar influence is neglected and therefore only mass transfers take place. In the presence of radiation, parameters like the light availability (both at the loop α_l and bubble column α_c), maximum photosynthesis rate, $P_{O_{2,max,t}}$, form parameters K_i and m_t, and the exponent, n_t, were adjusted using the dissolved oxygen, Eqs. (1) and (2), whereas mass transfer parameters for the bubble column (a_c and b_c, Eq. (22)) were calibrated by the difference between dissolved oxygen in the loop and in the bubble column, that is motivated by the influence of the air injection in the bubble column on the dissolved oxygen. On the other hand, respiration rate, Eq. (2), was established from results to 1% of the maximum photosynthesis rate. Furthermore, several measurements of biomass concentration were used to adjust biomass yield coefficient produced by the oxygen unit mass $Y_{o/x}$, Eqs. (4) and (5). The rest of parameters of Eq. (2) were maintained at values from laboratory scale, being necessary to perform aggressive test that limits the growth rate of the culture (close to limit conditions) to fit these parameters appropriately. Other parameters, such as tube diameter, culture heat capacity, tube length, etc., remained constants at values fixed by the design and previous knowledge of the system, and these parameters are shown in Tables 1 and 2.

Summarizing, the model is compound of two biological Eqs. (1) and (2), where a total of 14 characteristic parameters must be calibrated in real conditions, although six of them (z, K_{O_2}, B_1, C_1, B_2 and C_2), related to the factors of pH and dissolved oxygen, have remained at values from laboratory scale obtaining successful results. The rest of them (a_l, a_c, K_a, $P_{O_{2,max,t}}$, n_t, m_t, K_i, and r) have been calibrated by the procedure described in this work converging to an identifiable solution of these equations. Note that only the light availability parameter (a_l and a_c Eq. (1)) must be adjusted both for the solar receiver and for the bubble column due to the different solar exposition characteristics of each part. On the other hand, six mass balances (Eqs. (4),

Table 1 Common variables, constants, and characteristic parameters for both models

Parameter/Variable	Description	Value and units
B_1	Pre-exponential factors	2.4098
B_2	Pre-exponential factors	533.009
C_0	Drift flux model parameter	0.996
C_1	Activation energies	6.2684
C_2	Activation energies	68.8062
C_b	Biomass concentration	kg m^{-3}
$[CO_2^*]$	Equilibrium concentration with the gas phase for dioxide carbon	mol m^{-3}
$[CO_3^{2-}]$	Bicarbonate specie	mol m^{-3}
$[C_T]$	Total inorganic carbon concentration	mol m^{-3}
$[H^+]$	Hydrogen specie	mol m^{-3}
H_{CO_2}	Henry's constants for carbon dioxide	38.36 mol atm^{-1} m^{-3}
H_{O_2}	Henry's constants for oxygen	1.07 mol atm^{-1} m^{-3}
$[HCO_3^-]$	Carbonate specie	mol m^{-3}
I_{av}	Average solar irradiance	μE m^{-2} s^{-1}
I_0	Solar irradiance on an horizontal surface	μE m^{-2} s^{-1}
K_i	Form parameter	173.9504 μE m^{-2} s^{-1}
M_{CO_2}	Molecular weight of carbon dioxide	32 g mol^{-1}
M_{O_2}	Molecular weight of oxygen	44 g mol^{-1}
$[O_2]$	Dissolved oxygen concentration	mol m^{-3}
$[O_2^*]$	Equilibrium concentration with gas phase for oxygen	mol m^{-3}
Q_{gas}	Volumetric flow rate of gas	m^3 s^{-1}
Q_{liq}	Volumetric flow rate of liquid	m^3 s^{-1}
Q_m	Volumetric flow rate of culture medium	m^3 s^{-1}
t	Time	s
U_∞	Bubble accession rate	0.651 $m\ s^{-1}$
U_{gas}	Superficial velocity of the gas	0.0186 m s^{-1}
U_{liq}	Superficial velocity of liquid	0.0441 m s^{-1}
V_{mol}	Molar volume	20 L mol^{-1}
x	Longitudinal space along the loop	m
y_{CO_2}	Carbon dioxide molar fraction	
Y_{CO_2}	CO_2 to N_2 Molar ratio in gas phase	mol CO_2 (mol N_2)$^{-1}$
y_{O_2}	Oxygen molar fraction	
Y_{O_2}	O_2 to N_2 molar ratio in gas phase	mol O_2 (mol N_2)$^{-1}$
$Y_{o/x}$	Biomass yield coefficient	0.9713 kg $kg^{-1}O_2$
z	Form parameter	5.4333

Table 2 Variables, constants and characteristic parameters used into the tubular photobioreactor model

Parameter/Variable	Description	Value and units
a	Solar irradiance absorptivity	0.5411
a_c	Form parameters in the column	0.0806 s^{-1}
a_i	Interfacial area	m^{-1}
a_l	Form parameters in the loop	0.0012 s^{-1}
$A_{gas,l}$	Gas cross-sectional area of the loop	m^2
$A_{liq,l}$	Liquid cross-sectional area of the loop	m^2
$A_{t,c}$	Total cross-sectional area of the column	0.1257 m^2
$A_{t,l}$	Total cross-sectional area of the loop	0.0055 m^2
$A_{N_{2,l}}$	Cross-sectional area of the nitrogen gas in the loop	m^2
b_c	Form parameters in the column	0.7533
b_l	Form parameters in the loop	0.8450
$[CO_{2,p}]$	Carbon dioxide concentration in the liquid phase in the solar receiver	mol m^{-3}
$[C_T]_{m,t}$	Total inorganic carbon in the medium	8 mol m^{-3}
d_b	Bubble diameter	m
$d_{t,c}$	Total column diameter	0.4 m
$d_{t,l}$	Total loop diameter	0.084 m
$F_{N_{2,l}}$	Molar flow of nitrogen for gas phase in the loop	mol s^{-1}
$F_{N_{2,c}}$	Molar flow of nitrogen for gas phase in the bubble column	mol s^{-1}
$[HCO_3^-]$	Carbonate specie	mol m^{-3}
$K_{a,t}$	Extinction coefficient	133.0324 m^2 kg^{-1}
$K_{CO_{2,c}}$	Transfer coefficient constants for CO_2 in the column	0.91
$K_{CO_{2,l}}$	Transfer coefficient constants for CO_2 in the loop	0.91
K_l	Liquid-side mass transfer coefficient	m s^{-1}
$K_l a_{l,CO_2 c}$	Volumetric gas–liquid mass transfer coefficient for CO_2 in the column	s^{-1}
$K_l a_{l,CO_2 l}$	Volumetric gas–liquid mass transfer coefficient for CO_2 in the solar receiver	s^{-1}
$K_l a_{l,O_2 c}$	Volumetric gas–liquid mass transfer coefficient for O_2 in the column	s^{-1}
$K_l a_{l,O_2 l}$	Volumetric gas–liquid mass transfer coefficient for oxygen in the solar receiver	s^{-1}
$K_{O_{2,t}}$	Oxygen inhibition constant	0.7202 mol m^{-3}
m_t	Form parameter	0.0015
n_t	Form exponent	0.9779

(continued)

Table 2 (continued)

Parameter/Variable	Description	Value and units
$[O_2]_{m,t}$	Dissolved oxygen in the medium	0.2812 mol m^{-3}
$P_{CO_{2,t}}$	Carbon dioxide consumption rate	$kgCO_2$ kg^{-1} s^{-1}
$P_{O_{2,t}}$	Photosynthesis rate	kgO_2 kg^{-1} s^{-1}
$P_{O_{2,max,t}}$	Maximum photosynthesis rate	4.37E-05 kgO_2 kg^{-1} s^{-1}
P_T	Total pressure	1 atm
Q_w	Volumetric flow rate of water cross heat exchanger	m^3 s^{-1}
r	Respiration factor	0.01
V	Velocity of the fluid	1 m s^{-1}
$V_{liq,c}$	Liquid bubble column volume	m^3
$V_{gas,c}$	Gas bubble column volume	m^3
$V_{t,c}$	Total bubble column volume	0.4021 m^3
V_{ext}	Heat exchanger volume	20.3 L
$y_{N_{2,l}}$	Nitrogen molar fraction used in the solar receiver	
$y_{N_{2,c}}$	Nitrogen molar fraction used in the bubble column	
$Y_{o/x}$	Biomass yield coefficient	0.9713 kg $kg^{-1}O_2$
α_t	Distribution solar factor	
α_c	Distribution solar factor for bubble column	0.1052
α_l	Distribution solar factor for solar receiver	0.9725
ε_l	Gas holdup loop in the solar receiver	
ε_c	Gas holdup in the bubble column	

(6), (9), (11), (13), and (16)) for the solar receiver, and five mass balances (Eqs. (5), (8), (10), (12), and (14)) for the bubble column represented the temporal and spacial physicochemical phenomena that take place into the photobioreactors, where three parameters (a_l, b_l, and a) for the loop and two for the bubble column (a_c and b_c) have been calibrated.

Figure 5 shows some representative results of the calibration process, where both experimental and simulated concentrations of dissolved oxygen, pH, and biomass are shown. It can be seen how the model captures fast variations of the pH motivated by the CO_2 injections and the solar radiation, whereas smooth changes in dissolved oxygen and biomass concentration are also represented. As can be appreciated from Fig. 6, the model reproduces clearly the closed-loop nature of the system producing periodic oscillations related to the fluid velocity of the system, being one of the improvements reached with the use of PDE equations to model the transport phenomena respect to the model published in (Fernández et al. 2012). The calibration results showed mean errors between simulated and experimental data of 6.92 and

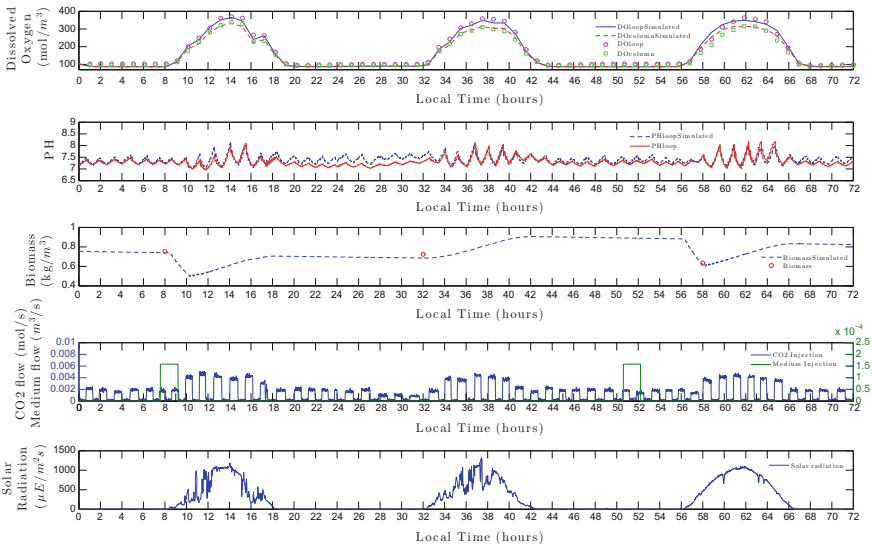

Fig. 5 Calibration results: Simulated and experimental data of dissolved oxygen concentration (DO), pH, and biomass concentration as a function of CO_2 injection and solar radiation (February 3, 4, and 5, 2014) (Fernández et al. 2014)

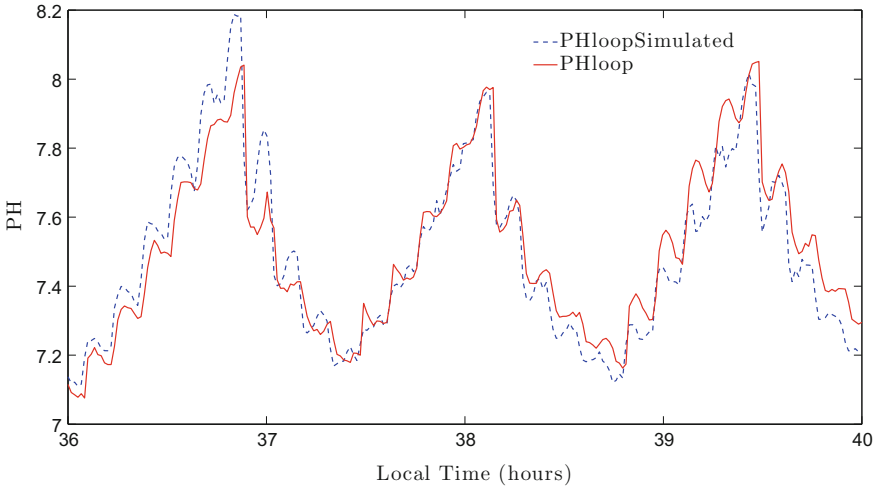

Fig. 6 Enlarged view of calibration results (Fernández et al. 2014)

4.99% for the dissolved oxygen (loop and bubble column, respectively), 1.65% for pH, and 3.44% for biomass concentration.

Figures 7 and 8 show similar results for the validation process, where an average of the parametric values obtained in the calibration process was considered (these

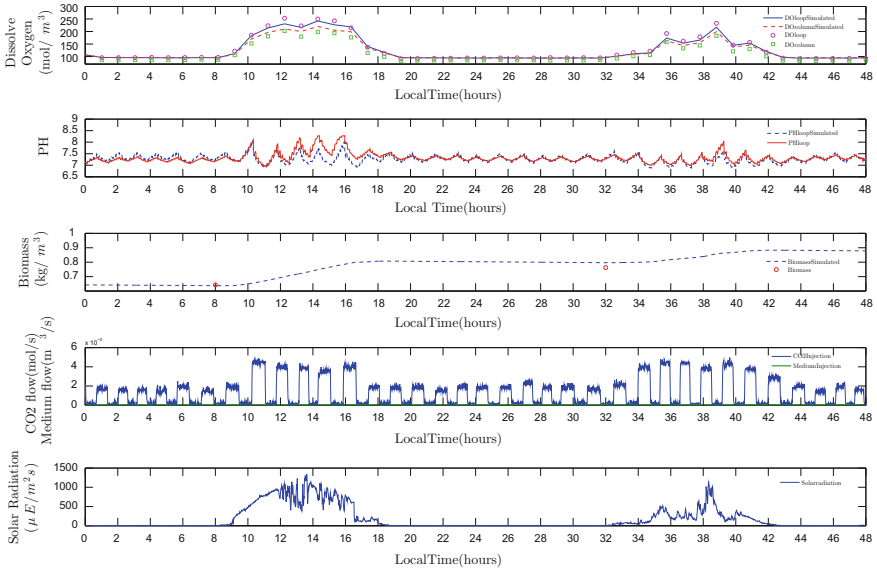

Fig. 7 Validation results: Simulated and experimental data of dissolved oxygen concentration (DO), pH, and biomass concentration as a function of CO_2 injection and solar radiation (February 25 and 26, 2014) (Fernández et al. 2014)

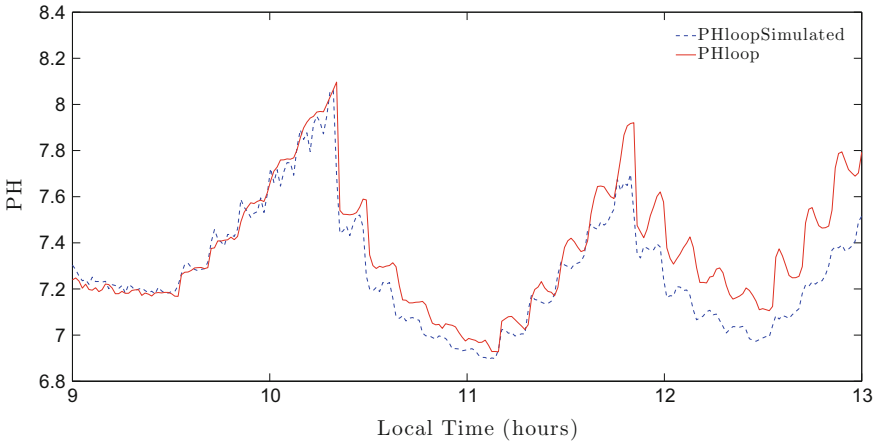

Fig. 8 Enlarged view of validation results (Fernández et al. 2014)

parameters are shown in Tables 1 and 2). The mean errors for this case were 3.43 and 10.81% for the dissolved oxygen, 1.56% for pH, and 2.81% for biomass concentration.

As observed from the calibration and validation results, the model properly fits the real data for the different process variables.

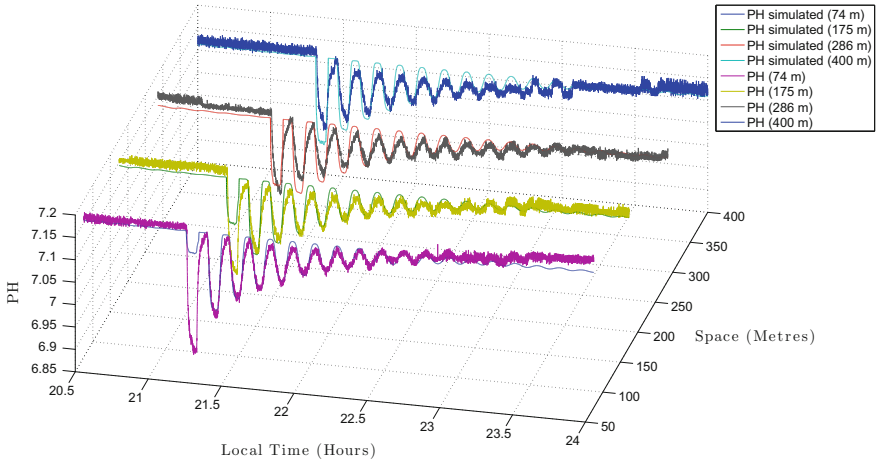

Fig. 9 Effects of a CO_2 pulse on the pH spatial distribution (Fernández et al. 2014)

4.1.2 Uses and Applications of the Model

In this section, some uses and applications are outlined. The previous results can be considered a good approximation to the main system dynamics from the input–output point of view. However, the model can be also used to analyze the behavior of the different variables along the loop in the reactor. Figure 9 shows a comparison between multiple measurement points located in different places of the loop. A CO_2 pulse was injected during the night (without solar irradiance) causing periodic oscillations due to the closed-loop nature of the system. It can be observed how the model reproduces this phenomenon, but the output of the model in the first cycle presents deviations from the real behavior mainly in the first sensors (those closer to the injection point). In view of these results, it can be concluded that in the first cycle, from the spatial point of view, the model should have to be improved by including molecular diffusion phenomena in the liquid phase to try to model this observed behavior (see Fernández et al. 2014).

On the other hand, the proposed model is able to predict the microalgal growth influenced by disturbances such as solar radiation and ambient temperature, which influence directly on the culture conditions (pH and dissolved oxygen). In addition to this, other system variables, which cannot be measured, are modeled such as carbon dioxide and total inorganic carbon concentration, oxygen, and carbon dioxide molar fraction in the gas phase, and even the carbon dioxide losses of the system. Moreover, relations between the culture conditions and the inputs broadly used in any kind of microalgal system are taken into account. The model can be considered as a useful tool in the optimization and design of photobioreactors, allowing to perform simulated studies for consecutive days both in discontinuous and continuous modes as can be seen in Fig. 7. Also, the proposed model can be used as a virtual sensor, allowing to predict unmeasured variables in a synchronous way to

the real plant and obtaining real-time estimations. From a control point of view, a dynamic first principle-based model provides a powerful tool to simulate any type of control strategies. Finally, since the model is based on physical, chemical, and biological principles, it can be also used to elaborate optimal or hierarchical control strategies. In these kinds of strategies, the problem is divided into layers where the upper layer is focused on the resolution of an optimization problem, while the lower layer manages the information provided from the upper layer in order to manipulate local regulators.

4.2 Results for Raceway Photobioreactor

In this section, the results for the raceway reactor are presented. The experimental data were obtained under normal operating conditions of the reactor (liquid velocity at 0.2 m s^{-1}, pH = 8 by injection of flue gas, semicontinuous operation at 0.2 day^{-1} dilution rate with volumetric flow rate of medium at 30 l min^{-1}) for different dates, covering a wide range of solar radiation conditions. Measurements of dissolved oxygen and pH at the end of each part of the reactor (channel, paddle wheel, and sump) were registered, in addition to environmental conditions (solar radiation, and temperature), and operation parameters (CO_2 injection, dilution of the culture). Initial values of characteristic parameters were obtained from previous knowledge. Thus, value of biological parameters such as the extinction coefficient ($K_{a,r}$), maximum photosynthesis rate ($P_{O_2,\max,r}$), form parameters (m_r, n_r, z), oxygen inhibition constants ($K_{O_2,r}$), pre-exponential factors (B_1, B_2), activation energies (C_1, C_2), and the respiration constant (R_{O_2}) were taken from (Costache et al. 2013). On the other hand, values of mass transfer coefficients for each part of the reactor were taken from Godos et al. (2014) and Mendoza et al. (2013b).

4.2.1 Model Calibration and Validation

To determine the real values of these characteristic parameters through the calibration and validation processes, data of dissolved oxygen and pH from the real reactor were compared with the output of the model using an optimization problem on the IAE error, an optimum value of these parameters being determined for each day used in the calibration procedure. Figure 10 allows to compare the real and simulated data obtained using the developed model. As observed, the model response fits the experimental data for both dissolved oxygen and pH. The model captures smooth variations of the photosynthetic rate produced by the bell-shaped form of the solar radiation. Increase of dissolved oxygen and pH due to photosynthesis by solar radiation availability is simulated. Changes produced through CO_2 injections, as pH and dissolved oxygen decrease, are also represented by the proposed model, taking into account the different dynamics taking place with and without the presence of solar radiation. Moreover, the model allows us observing how the characteristic delay of

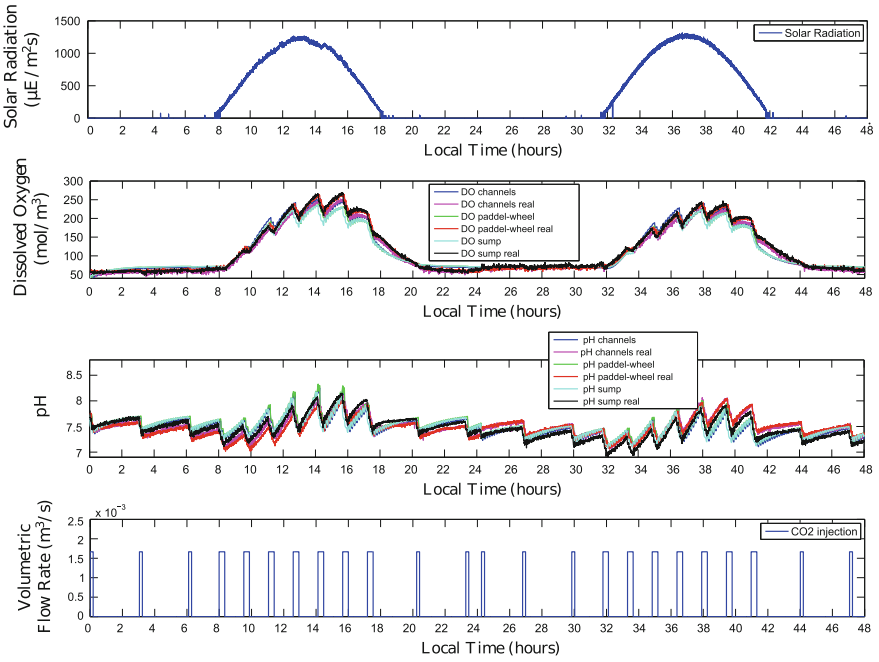

Fig. 10 Experimental and simulated data of dissolved oxygen concentration and pH at the end of the channels, paddle wheel, and sump. All of them as a function of CO_2 injection and solar radiation of two representative days used for model calibration purposes (Fernández et al. 2016)

the system produces an increase of pH and dissolved oxygen in the channels and paddle wheel, both variables decreasing after the sump. The mean errors between the simulated and experimental data were of 8.2, 8.6, and 10.0% for the dissolved oxygen at the end of the channel, paddle wheel, and sump, respectively, and of 1.5, 1.8, and 1.6% for the pH for the same sections.

As a result of the calibration procedure, the average values of characteristic parameters were determined (see Tables 1 and 3). Regarding biological parameters, the maximum photosynthesis rate was 2.06×10^{-5} kgO_2 kg^{-1} s^{-1}, whereas the extinction coefficient was 80 m^2 kg^{-1}, and the coefficients respiration rate (R_{O_2} and R_{CO_2}) were 9.58×10^{-7} kgO_2 kg^{-1} s^{-1} and 4.28×10^{-6} $kgCO_2$ kg^{-1} s^{-1} for oxygen and carbon dioxide, respectively. The rest of biological parameters being $n_r = 1.045$, $K_i = 174$ μ E m^{-2} s^{-1} and $m_r = 0.0021$. Regarding the volumetric mass transfer coefficients for oxygen in the different sections of the reactor, the values obtained were 2.5×10^{-6} s^{-1}, 2.2×10^{-3} s^{-1} and 6.3×10^{-3} s^{-1}, for channel, paddle wheel, and sump, respectively.

Validation of the model was performed to check the model quality, using a different set of data in different dates (validation data), in order to avoid possible bias and variance errors. For this validation process, the reactor was operated under the same conditions that in the calibration stage, where model includes the characteristic parameters obtained from calibration process being compared with experimental

Table 3 Variables, constants and characteristic parameters used into the raceway photobioreactor model

Parameter/Variable	Description	Value and units
$[CO_2]$	Carbon dioxide concentration in the liquid phase	mol m^{-3}
$[C_T]_{m,r}$	Total inorganic carbon in the medium	3 mol m^{-3}
h	Liquid height	0.17 m
h_w	Wall height	0.46 m
h_s	Solar hour	h
h_{ss}	Subterranean height of the sump	h
$K_{a,r}$	Extinction coefficient	80 m^2 kg^{-1}
$K_{laO_{2_{ch}}}$	Volumetric gas–liquid mass transfer coefficient for CO_2 in the channels	$2.5000 \cdot 10^{-6}$ s^{-1}
$K_{laO_{2_p}}$	Volumetric gas–liquid mass transfer coefficient for CO_2 in the paddle wheel	0.0219 s^{-1}
$K_{laO_{2_s}}$	Volumetric gas–liquid mass transfer coefficient for CO_2 in the sump	0.0063 s^{-1}
$K_{laCO_{2_{ch}}}$	Volumetric gas–liquid mass transfer coefficient for CO_2 in the channels	s^{-1}
$K_{laCO_{2_p}}$	Volumetric gas–liquid mass transfer coefficient for CO_2 in the paddle wheel	s^{-1}
$K_{laCO_{2_s}}$	Volumetric gas–liquid mass transfer coefficient for CO_2 in the sump	s^{-1}
$K_{O_2,r}$	Oxygen inhibition constant	0.8373 mol m^{-3}
l_s	Length of the sump	1 m
m_r	Form parameter	0.0021
N	Day of the year	d
n_r	Form exponent	1.045
$[O_2]_{m,r}$	Dissolved oxygen in the medium	0.2812 mol m^{-3}
P_b	Net production of biomass	kgO_2 kg^{-1}
$P_{CO_{2,r}}$	Carbon dioxide consumption rate	$kgCO_2$ kg^{-1} s^{-1}
$P_{O_{2,r}}$	Photosynthesis rate	kgO_2 kg^{-1} s^{-1}
$P_{O_{2,max,r}}$	Maximum photosynthesis rate	$2.06 \cdot 10^{-5}$ kgO_2 kg^{-1} s^{-1}
R_{O_2}	Respiration coefficient for dissolved oxygen	$9.58 \cdot 10^{-7}$ $kgCO_2$ kg^{-1} s^{-1}
Q_h	Volumetric flow rate during the harvesting process	m^3 s^{-1}
s_x	Shadow onto the surface of the cross-sectional area	m^2
V_r	Velocity of the fluid	0.2 m s^{-1}
V_p	Volume of the paddle wheel	0.1651 m^3
V_s	Volume of the sump	0.8151 m^3
w	Width of the channel	1 m

(continued)

Table 3 (continued)

Parameter/Variable	Description	Value and units
w_s	Width of the sump	0.9 m
y_{N_2}	Nitrogen molar fraction	
Y_{b/O_2}	Biomass yield coefficient	0.7273 kg
ε_s	Gas holdup in the sump	
α_s	Distributed factor	
α	Solar altitude angle	
δ	Sun declination	
ω	Solar hour angle	
ϕ	Latitude	
γ	Azimuth angle	
γ_0	Angle from North to the normal vector of the reactor	

data. Figure 11 shows how the simulation fits experimental results, the adequacy of the model to simulate experimental data being confirmed. The mean error for the dissolved oxygen was 4.9, 1.6, and 1.5% at the end of the channel, paddle wheel, and sump. On the other hand, the difference between the experimental pH of the culture and the simulated pH was 1.1, 1.0, and 1.2% for the same sections, proving an accurate response of the model for real conditions of operation.

It can be concluded that the proposed model captures the variations of the dissolved oxygen caused by the daily variation of solar radiation, and consequently of the photosynthesis rate. Furthermore, the model represents the transfer and consumption of carbon dioxide, thus allowing to simulate the variation of pH as a function of photosynthesis rate and CO_2 injections performed to feed the system and control the pH of the culture.

4.2.2 Uses and Application of the Model

Once the model has been calibrated and validated, it can be also used to simulate different designs, conditions, or scenarios (Fernández et al. 2016). For instance, the influence of the height of the reactor wall on the light availability can be analyzed. Figure 12 shows the influence of normal wall height of the reactor (h/h_{liq}) demonstrating that using walls with heights double than liquid reduces the productivity a 30%. As was expected, the optimal point was found in the unit of this relation, since on the contrary the difference between these heights generates shadows when the solar radiation is projected onto the surface of the liquid, producing a lower level of production. Once the wall height is optimized, the other relevant parameter is the water depth because it determines the light availability inside the culture through the average irradiance. Figure 13 shows the results obtained modifying the liquid height

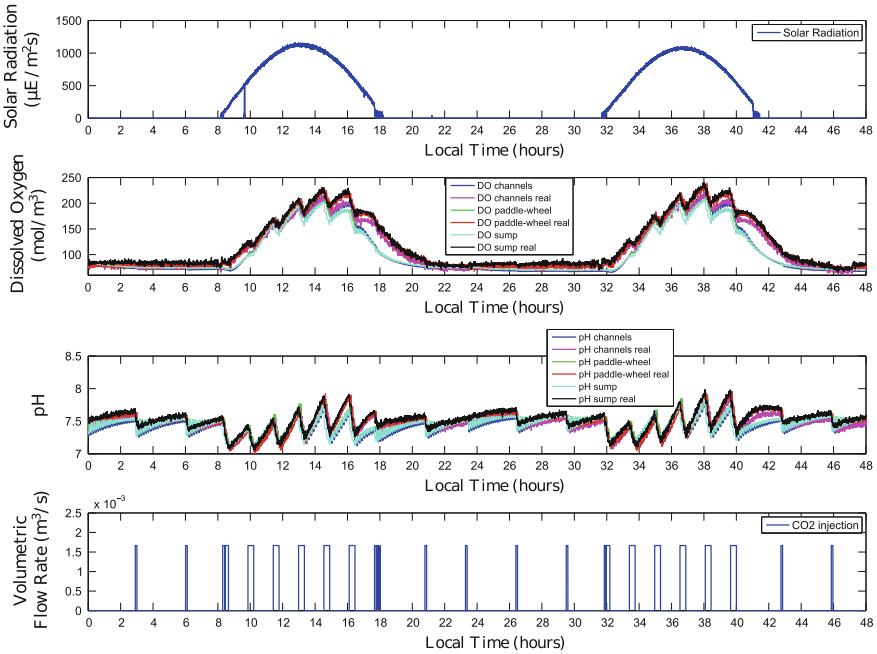

Fig. 11 Experimental and simulated data of dissolved oxygen concentration and pH as a function of CO_2 injection and solar radiation of two representative days used for validation purposes (Fernández et al. 2016)

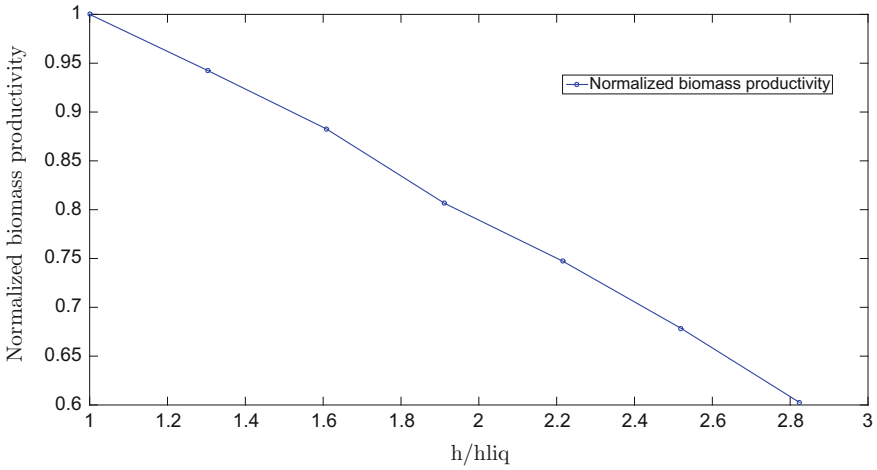

Fig. 12 Influence of normalized wall height on normalized biomass productivity of *Scenedesmus almeriensis* semicontinuous cultures in outdoor raceway reactor under standard conditions (Fernández et al. 2016)

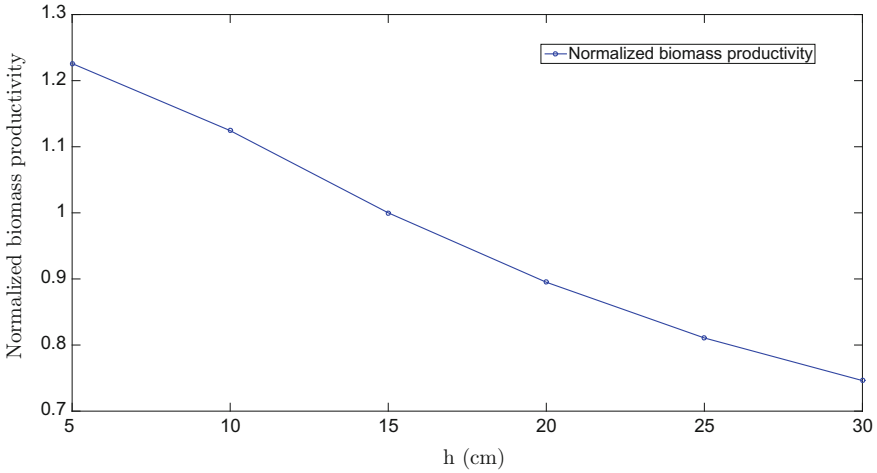

Fig. 13 Influence of water depth on normalized biomass productivity of *Scenedesmus almeriensis* semicontinuous cultures in outdoor raceway reactor under standard conditions (Fernández et al. 2016)

into the reactor but maintaining the rest of operational and design conditions (length and wide) of the reactor in any case. Note that in this case, the wall height and the liquid height have been established to equal values in order to avoid the negative effects produced by shadows in the system. Data show how the biomass productivity of the system exponentially increases when reducing the water depth, increasing 72% when the water depth reduces from 30 to 5 cm. It is important to note that these results do not consider the difficulty of operating large raceways at low water depth, as losses of efficiency on the paddle wheel or water depth variations along the reactor. Some other analysis, as the channel length, among others, can be done. See (Fernández et al. 2016) for a detailed analysis.

On the other hand, the developed model is a powerful tool for the study of existing raceway reactors producing microalgae biomass. Thus, from direct measurements of dissolved oxygen, pH, and CO_2 injection, it is possible to obtain the values of characteristic parameters of the system, both biological and engineering ones. Data obtained from calibration of the model versus experimental data agree with previously reported for this reactor (Godos et al. 2014; Mendoza et al. 2013a, b). The extinction coefficient of the biomass is in the range of 50–200 $m^2\ kg^{-1}$ reported for microalgae, whereas the photosynthesis rate is more than one order of magnitude higher than the respiration rate of $2.06 \cdot 10^{-5}$ and $9.58 \cdot 10^{-7}$ $kgO_2\ kg^{-1}\ s^{-1}$, respectively. The model also allows us to determine characteristic parameters of the growth model of the strain used (n_r, K_i, and m_r) that are usually determined at laboratory conditions, requiring long time and numerous experiments (Costache et al. 2013). Regarding the mass transfer coefficients determined from the calibration procedure, results agree with previously reported for the same reactor (Mendoza et al. 2013a), confirming that mass transfer mainly takes place in sump and paddle wheel. The

model confirms that channels perform as a "tubular reactor", where no relevant exchange of oxygen and CO_2 takes place between the culture and the atmosphere, besides the general knowledge that in raceway reactors oxygen accumulation does not take place and large CO_2 losses take place into the channel. Thus, the model allows us to demonstrate that oxygen is accumulated into the culture up to values of 0.4 mol/m^3 because the photosynthesis rate is higher than oxygen desorption capacity. On the opposite, the injected CO_2 is higher than CO_2 consumption by the cells, being mainly lost to the atmosphere into the sump and paddle wheel. However, CO_2 losses into the channels are three orders of magnitude lower. This behavior is analogous to that reported in tubular photobioreactors (Camacho et al. 1999). Then, it can be concluded that the design of both types of reactors, raceway and tubular, is not so different as usually reported.

Another interesting usage of the modes is to see that results here reported concerning the spatial–temporal variation of culture parameters demonstrate that static models cannot be used to adequately represent this type of reactors. Thus, microalgae have different responses (photosynthesis rate, etc.) to changes in the culture conditions not only along the day but also at the different positions inside the reactor (Fernández et al. 2012). Several studies reported dissolved oxygen concentrations in raceways as high as 500 %Sat., causing inhibition of photosynthesis and growth, and eventually leading to culture death (Marquez et al. 1995; Mendoza et al. 2013b; Singh et al. 1995; Vonshak 1997). Otherwise, it has also been reported that algal cultures in raceways can become carbon limited if only CO_2 from the air is available (Stepan et al. 2002), to maximize the productivity being necessary to maintain a CO_2 concentration in the bulk liquid of at least 65 μmol/L and a pH of 8.5 for high productivity of some microalgae (Weissman et al. 1988). To provide CO_2 and maintain the pH, it is possible to supply flue gases, but it is necessary to adequately design and operate the CO_2 supply unit (Godos et al. 2014). Moreover, the existence of pH gradients into the reactor reduces the performance of microalgae cultures (Berenguel et al. 2004; García et al. 2003). Experimental data here reported confirm the existence of relevant variations of culture conditions with time and position inside the reactor. Variation of culture parameters along the day is a consequence of solar daily variation, whereas variations along the different sections of the raceway is a consequence of the different rates of phenomena taking place (biological, physic, and chemical).

5 Conclusions

Two dynamic models based on first principles of the production of microalgae in raceway and tubular reactors have been developed, calibrated, and validated. The developed models have demonstrated to reproduce the spatial and temporal variations of main variables (light, biomass, dissolved oxygen, carbon dioxide, and pH) in tubular and raceway reactors, where biological and engineering aspects of the system are integrated. These models are useful tools to design and operate photo-

bioreactors in a conservative way, for the boundary conditions of maximum solar radiation availability or whatever other situation. However, as the model also allows us to include the variation with time of culture parameters, it can be used for the implementation of advanced control strategies, and to refine the design and operation of open reactors taking into account the dynamic accumulation or uptake of compounds, thus optimizing it. Dynamic models based on first principles as those here reported are a necessary and powerful tool for the improvement of industrial reactors.

Acknowledgements This work has been partially funded by the following projects: DPI2014-55932-C2-1-R (Spanish Ministry of Ecomony and Competitiveness and FEDER funds); EDAR-SOL CTQ2014-57293-C3 (Spanish Ministry of Science and Innovation); PURALGA RTA2013-0056-C03 (INIA), and supported by Cajamar Foundation.

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Generation and Harvesting of Microalgae Biomass for Biofuel Production

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Abstract Microalgae are considered one of the promising and favorable feedstocks for biofuel production and suggested as potential natural sources of functional ingredients of food and pharmaceuticals. However, currently available energy-intensive harvesting strategies have been considered as major bottlenecks of microalgal industry for economic production of fuels and value-added products. Harvesting by centrifugation and filtration are generally characterized as high cost and energy capture method which comprises above 20% of total production cost in algae industry. Flocculation is accounted as most economically competitive and effective approach for microalgal biomass harvest at large scales compared to other access methods. In this chapter, we present various microalgal harvesting technologies with a focus on environment gracious bioflocculation methods. This is practically true that, harvesting of microalgae through the bioflocculation does not involve much capital investment, and moreover, energy consumption associated with the bioflocculation process is negligible, thus having a key position to reduce the production cost, but paradoxically are less studied than the other chemical and physical flocculation. This chapter also provided the new idea of infochemical-based flocculation and information about the future promising genetic modified microalgae with flocculation phenotype to harvest microalgae cells from culture medium. In addition, the concise overview of the mechanism behind these technologies and their possible solution for overcoming the existing challenges to improve the efficiencies of flocculation was highlighted.

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B.N. Tripathi and D. Kumar (eds.), *Prospects and Challenges in Algal Biotechnology*, DOI 10.1007/978-981-10-1950-0_3

1 Introduction

The world is facing various challenges to provide sufficient food and energy supply to the increasing population, to protect the environment from degradation and ensure proper human and animal health associated with climate change. The potential threat of global climate changes is increasing that has been credited to greenhouse gas (GHG) emissions from fossil fuel use (Alam 2016a; Suganya et al. 2016; Waltz 2009; Wijffels et al. 2010). In addition, the consumption of liquid biofuels in modern transportation sectors has shown swift global demand in the recent years and provoked repeatedly by policy maker by for accomplishment of energy security, and alleviation of uprising greenhouse gase (e.g. CO_2) emissions to the atmosphere (Milano et al. 2016) to ensure the sustainable environment. From energy outlooks, think tanks have estimated that the growth in production and consumption of biofuel will continue further in future, but their influences towards meeting the overall energy demands will remain limited due to: deficiency of well-managed transportation in underdeveloped countries, shortage of well-managed agricultural practices with the mounting economies, high water and fertilizer requirements for crops production, and required preservation of bio-diversity (Louw et al. 2016; Rawat et al. 2011). Technically and economically feasible conditions of biofuel resource are (Borowitzka 2016; Brennan and Owende 2010): biofuel should be reasonable or lower in cost than gasoline fuels; should require minimum agricultural and forest land; should assist for the quality improvement of air (e.g. CO_x and NO_x sequestration), and should reduce fresh water use. Cautious management of culture system for microalgae biomass generation could meet these conditions and therefore make a very important contribution to meeting the crucial clean energy demand, as well as acting as cell factories for the production of numerous value-added functional food and pharmaceutical ingredients, and recombinants proteins (Ibañez and Cifuentes 2013; Rawat et al. 2011) while simultaneously providing environmental benefits by bioremediation of waste water (Alam et al. 2015).

Microalgae are one of the most promising bioresource that are receiving a pointedly accelerated priority with the increasing recognition of their potential ability to produce biofuel, diverse functional ingredients of food and pharmaceuticals and for their unique role in bioremediation and synthetic biology research (Alam et al. 2015; Ibanez et al. 2013). Therefore, there has been a vast amount of attention in developing algal biofuel production systems as a way of taking on the challenges of climate change and decreasing fossil fuel reserves. A considerable number of R&D is ongoing in this area, in various countries by both governmental agencies and private enterprise cooperated with both at the academic fundamental level and the industrial-applied level. To produce biofuel from microalgae is of course already technically possible, but it is necessary to reduce their costs as it is relatively expensive compared with petroleum fuels due to the lack of economically feasible technology, specially in downstream processing. Thus, it is essential to resolve many of bottlenecks from algal culture for fuel production allied with

economical, engineering, and biological aspects to reduce the production costs at modest scale (Chisti 2013; Wan et al. 2015). To rank the degree of influence of each of the factors that are hurdles to commercial production of microalgal biofuel are high initial investment, harvesting of microalgae from diluted medium, and desired biomass productivity with high values contents. Current decade has seen an explosion in research and development (R&D) for increasing the yield of microalgal biomass and lipid production through modern open culture technique and photobioreactor design (Chen et al. 2011), selection of ideal strains (Borowitzka 2013; Larkum et al. 2012), genetic engineering of metabolic pathways for value-added coproducts (Shang et al. 2016), new approach of lipid and biodiesel extraction (Lu et al. 2016; Pan et al. 2017). However, less progress has been achieved on research and innovation in downstream processing, although this is crucial to reduce the cost of the biofuel production process. The leading challenge in downstream processing of microalgae biofuel lies in unraveling/collecting the microalgae biomass from diluted growth medium, which is known as harvesting process. Usually the concentrations of biomass in microalgal cultures are low (ranging between 0.02 and 0.05% biomass), because the high biomass concentration directed the reduction in productivity; therefore, a huge volume of water needs to be removed to collect the biomass; thus the energy input for this step can represent a major part of the total energy contribution. The challenge is to concentrate cells from a dilute solution through either one or more physical, chemical, or biological steps either in a combined or separate way. Familiar harvesting techniques include filtration, flotation, sedimentation, and centrifugation. However, there is no particular harvesting method suitable for every strain. The choice of the harvesting procedure is mostly reliant on the microalgal properties such as density, size, and the ultimate market value of the preferred product (Rawat et al. 2011; Brennan and Owende 2010). For cost-effective product development, it is obvious that harvesting methods should be tailored according to the properties of the specific microalga species that will be harvested, and the cost of the harvesting method needs to consider against the value of the end-product.

Membrane filtration technology has been progressively applied in microalgae industries to separate microalgae from fresh or seawater with a long history. At present in filtration technique, numerous filters such as plate-and-frame pressure filter, dynamic microfilter, rotary drum filter, tangential flow filter vacuum filter, etc., have been used for microalgae dewatering and collecting for small-scale cultures with closed photobioreactor for the production of value-added products, in which much higher biomass density could be achieved (Mo et al. 2015; Shao et al. 2015). In practice, either small or large scale, centrifugation is the most usable method for harvesting microalgae to date. Centrifugation technique with disk stack centrifuges is applied for microalgae harvest during large-scale processing, but significant capital investment in the equipment and energy makes this technology expensive and it is not economical for low value-added product such as biofuel

(Barros et al. 2015; Grima et al. 2003). In addition, unwanted material can also be concentrated up during centrifugation process and they have risk of damaging the cells, because of high shear forces that causes a loss of biomass and value-added substances. Biomass recovery by flocculation specifically by bioflocculation of microalgal cells does not involve huge capital investment, considering bioflocculation process as one of the most economically viable and innovative strategies for microalgal biomass harvest at large scales (Alam et al. 2016b). Today, microalgal-related products production are rapidly moving from laboratory to pilot-scale and commercial-scale demo installations (Rawat et al. 2011), provoking the necessity for cost and energy-efficient downstream processing technologies to make the industry economically competitive. Advances in the method of separating the algal biomass from the water will improve the prospects of algae oil and other value-added products. Microalgal flocculation processes induced by various types of microorganisms (self-flocculating algae, bacteria, fungi, or yeast), by extracellular polymer substances, or by bioflocculants produced by algae or bacteria (also known as flocculating agents) are often referred to as bioflocculation methods (Alam et al. 2016b; Alam et al. 2014; Wan et al. 2015). This type of flocculation was described a few decades ago as a potential environment-friendly harvesting procedure (Lavoie and De la Noüe 1987). However, bioflocculation is currently not widely applied in harvesting processes, because the induction mechanisms are poorly understood.

2 Microalgae

Microalgae are recognized as one of the oldest autotrophic primitive microorganisms of plant (thallophytes) life taxa in the world, have no roots, stems and leaves, have chlorophyll *a and b* as major photosynthetic pigments and can process simple reproductive structure with rapid growth rate (Bowman 2013; Umen 2014). The microalgal cells produce three key biochemical components by de novo synthesis such as lipids (oil), carbohydrates, and proteins. It is well known that, microalgae is able to synthesis and can rapidly gather higher amounts (10–100 times) of lipids than terrestrial oil plants due to their high growth rates. However, the lipid yields and growth rates of microalgae significantly vary among different species as well as specific culture environment. Algae structures are largely for energy conversion without any development or alteration the cells, and their simple development allows algae cells to adapt to prevailing and adverse environmental conditions and grow and accumulate in the long term for biofuel production (Bowman 2013) (Fig. 1).

Fig. 1 Microalgae culture facilities of Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences (GIEC-CAS), China. Flat plate photobioreactors (up-left), column photobioreactors (up-right), open pond culture (down)

3 Microalgae Harvesting Methods

Almost all microalgal biomass are cultured in diluted medium; thus it is required to separate the biomass from diluted broth as the molecule of interest is stored in the microalgal cells that are used for production of biodiesel and other coproducts. This process requires one or more solid–liquid separation steps. The choice of harvesting technique depends on the feature of microalgae, such as size, shape, concentration, and the value of end products (Brennan and Owende 2010). The choice of harvesting technology is fundamental to profitable production of microalgal biomass due to its potentially extreme energy usage that may account to more than 20% of the total costs of biofuel and other value-added food and chemical production. However, there is no specific harvesting method appropriate for all microalgae species. Microalgae harvesting is mainly divided into two steps process, specifically

1. Bulk harvesting—the purpose of bulk harvesting is to separate microalgal cells from diluted suspension as much as possible through flocculation or filtration. The main influencing factor of this technique is an initial biomass concentration in the suspension.

2. Thickening—the purpose of thickening is to concentrate the algae slurry by applying centrifugation process. Thickening is more energy-demanding process than bulk harvesting.

3.1 Settling/Sedimentation/Gravity Sedimentation

Sedimentation or gravity sedimentation is the simplest process of solid–liquid separation used for a long time which can separate a feed suspension into a slurry of higher concentration and an effluent of substantially clear liquid (Allnutt and Kessler 2015; Brennan and Owende 2010). This technique has been applied for harvesting of numerous microalgae strains and has widely been used in wastewater treatment plant, where the high bacterial load and nutrient levels influence to favor tramping and settling the microalgae. Cell density and the size and shape of the algal cells influence its utility as well as flow rate of the process of sedimentation, which has been enhanced by lamellar separators (plate) and sedimentation tanks. The sedimentation rate is controlled by the net force acting on the microalgae cells and this phenomenon is qualitatively described by Stokers Low (Pahl et al. 2013). Sedimentation process depends on various factors such as growth stage, size, shape, and density of microalgae cells. The sedimentation rate of microalgae can also depend on cell motility, growth medium turbulence, and water upwelling (due to temperature, wind stratification, and interference with other settling bodies). Flocculants or settling accelerant can be added in order to enhance the microalgal particle size and density to quicken the rate of sedimentation and separation. Sedimentation is a slowest separation process and less useful for industrial operation. However, the main benefits of sedimentation processes are low or no power consumption, low cost of process design, and low requirement for skilled operators (Fig. 2).

3.2 Filtration and Screening

Filtration and screening processes use permeable medium to separate solids from liquids. In this process, microalgae cells retain into the screen and allow the culture medium to pass through without excessive clogging. Filtration and screening systems depend on the microalgae being retained based on their shape and size, whereas attachment is facilitated by the physical, electrical, or chemical interactions between the microalgae cells and culture medium.

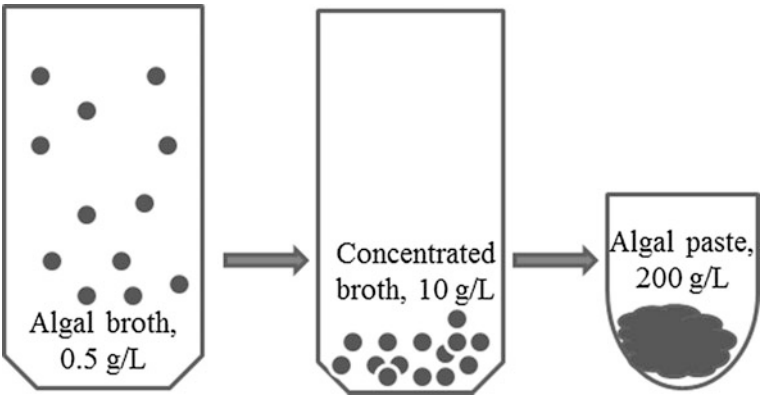

Fig. 2 Two-steps process for economical harvesting of microalgae. In the first step, diluted microalgae cells suspension (0.5 g/L) is pre-concentrated by flocculation or sedimentation or flotation to achieve 20 times concentration slurry with a biomass concentration of 10 g/L. Microalgal biomass is then further dewatered using a mechanical dewatering method such as centrifugation to achieved microalgal paste of 200 g/L

3.2.1 Screening

Screening process allows culture medium to pass onto a screen of given slit size. The microalgae cells collect according to desired size with given pore of screen. This harvesting method is applicable for a limited number of algae strains that are filamentous and large in size such as *Spirulina* and Dortmund *Coelastrum*. Unfortunately, most microalgal strains currently utilized are tiny in size and non-filamentous and not suitable to harvest in this manner (Allnutt and Kessler 2015). *Chlorella* sp. cannot be harvested with microstrainers filters. During microalgae harvesting, two screening techniques named vibrating screen filters and microstrainers are extensively used. Microstrainers consist of rotary drum and collect the particles with frequent backwash spray processes. Simple function, easy operation, low investment, low energy consumption, and high filtration ratio are main advantages of this process whereas buildup of bacterial and algal slime on the microfabric is the main drawback of this process. A high concentration of microalgal cells can block the screen, whereas a lower concentration of microalgae is insufficient for capture in this process.

3.2.2 Filtration

All filtration involves a pressure drop to be functional across the medium to provide force into fluid to flow through the filter and this operation can be continuous or discontinuous. According to the required magnitude of medium determines one or more of the following dynamic force may use: gravity, vacuum, pressure or centrifugal. There are two basic types of filtrations such as surface filters and depth

filters/deep bed filtration that are used. In surface filters, algae cells are deposited in the form of a cake on the face of a thin filter medium whereas algae cells are deposited within the filter medium in deep bed filtration. Major drawback of filtration is the requirement of frequent backwashing, which decreases the concentration of microalgae.

Filter Presses

Various press filtration methods have been tested by researcher with varying degrees of success. Mohan et al. tested five different pressure filters namely Chember filter press, Belt press, Pressure Suction filter, Cylindric Sieve, and Filter Basket for *Colastrum* harvesting: (Mohn 1980). A filter press consisting of a set of plates interspaced with filter or culture media. It utilizes a dead end filtration process where the suspension is pumped into each press compartment and flows vertical to the filter media. Microalgae cells retained by the filter media build up to form a cake. When the microalgae cake consumes the void between the plates, the filter press is full and filtration ceases. The filtration process must be temporarily stopped time to time to allow the plates being separated and the algae cake discharged. While filter presses can be automated to minimize operator requirements, they are infrequently used to recover microalgae.

Tangential Flow Filtration

Tangential flow filtration is very promising for the recovery of microalgae biomass from large volumes of culture. The advantages of tangential flow filtration over other recovery techniques is that better filtration rates can be attained to completely collect debris and microalgal cells. Tangential flow filtration using ultra filtration (typically 1–100 nm pore size) or micro-filtration (typically 100–10,000 nm pore size) membrane technology can be used to retain particles and several studies conducted by Danquah et al. (2009) and Stevens et al. (2013), and have successfully harvested microalgae using this technology.

Vacuum Filters

The most commonly used vacuum filters are the Vacuum-leaf filter and Vacuum-Nutsche filter. Both filters are inexpensive and very easy to use, and can cope with frequent changes in process condition. However, the rotary-vacuum-drum filter or RVDF was the most popular vacuum filter few years

ago. This filter can also be used for harvesting very tiny microalgae such as *Scenedesmas* sp. (Vigneswaran 1989). By using vacuum filters, majority of algae can be flocculated with algal paste concentration of 0.5–5%, where the water removal rate is normally 40–60% with algal biomass recovery rate over 90% (Pers. Comm. Parkson Corporation).

Gravity Belt Filters

Gravity belt filters are one of the most usable filters which consist of a fabric mesh (filter media) that moves over rollers driven by an adjustable speed or motion. They are continuously operating device which thicken microalgae biomass by gravity on a revolving porous filter belt. This filter use to those microalgae biomass which already been primary concentrated or flocculated to increase the particle size and dewater ability. Particle thickening occurs by gravity drainage of medium through the fabric mesh. Gravity belt filters generally compromise low capital cost and low power consumption. While the final water content in microalgae pest can be controlled to some extent by varying the water drainage time on the belt, the degree of thickening is dependent on the floc characteristics and the way that the water bounded to the slurry of algal biomass. Gravity belt filters are often designed for a maximum 5–7% solids concentration.

3.3 Flotation

Flotation is a another type of gravity separation method based on the attachment of air or gas bubbles to microalgae cells and the air bubble moves the microalgae cells to the surface from where microalgae cells can be collected. In this harvesting technique, microalga cells are carried to the surface of the medium and accumulated as floating substances. The success of flotation rate depends on the instability of the microalgae cells in the medium. The lower the instability, the higher the instability contacts. Most of the flotation methods can halt particles that are less than 550 μm, making these methods particularly suitable for unicellular microalgae (Laamanen et al. 2016). The flotations are classified according to the way of bubble generation such as dissolved air flotation, dispersed air flotation, ozone flotation, and electrolytic flotation, etc.

3.3.1 Dissolved Air Flotation (DAF)

Dissolved air flotation separates microalgae from its culture medium using features of both froth flotation and flocculation. It uses chemicals to flocculate microalgae with fine bubbles supplied by an air compressor (Lakghomi et al. 2015). This is a well developed and widely used lab-scale method and has already been scaled up in

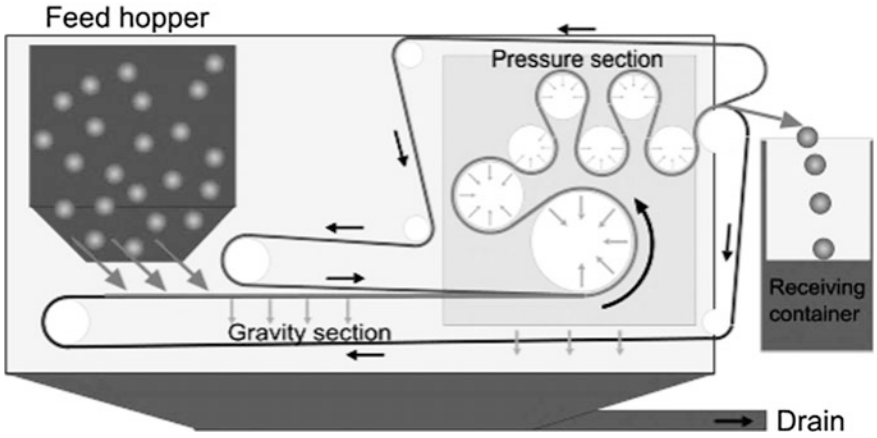

Fig. 3 Cross-sectional view of gravity belt filters; source: Wikiwayman at Wikipedia

large volumes for commercial microalgal biofuel production. The dissolved air flotation units use a compressor to achieve supersaturate flotation water with air in a saturator. The flotation water is then released into a flotation cell at atmospheric pressure, causing air to precipitate as small bubbles from the solution. Under moderate shear, bubbles adhere to the microalgal cells and cause them to float to the medium surface. The operational costs of DAF systems are generally higher than sedimentation due to the high energy demand for supersaturating the water with supplying air under expected pressure. However, another main drawback of DAF is that it suffers from the need of additional chemicals such as aluminum, chromium, potassium, sodium, or iron to the process that needs to be dealt within downstream processing which is expensive as well as not environmental friendly (Ndikubwimana et al. 2016). A typical cross-sectional view of a DAF unit is presented in Fig. 3.

The biomass harvesting clarification degree depends on various operational parameters such as: air tank pressure, hydraulic retention time, and microalgal cells flotation rate, while sedimentation concentration depends on the skimmer velocity and its height above medium surface (Kitchener and Gochin 1981; Lakghomi et al. 2015) and it is possible to obtain microalgal slurries of up to 7% compared to 2–3% for sedimentation (Ndikubwimana et al. 2016).

3.3.2 Dispersed/Suspended Air Flotation

Dispersed or suspended air flotation is similar to dissolved air flotation with respect to mechanism which creates small bubbles that adhere to microalgae cells, forcing them to the stay at the surface of the medium. Dispersed air flotation units create

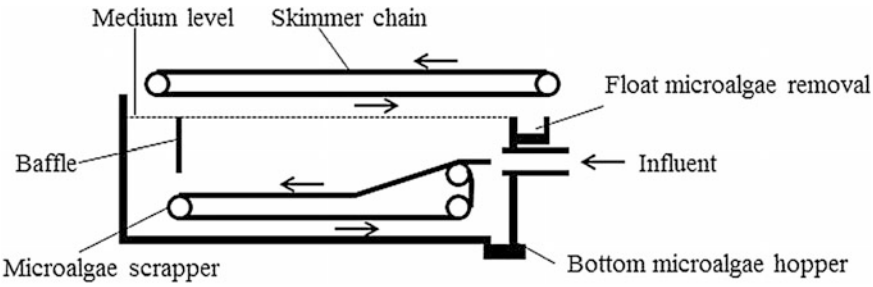

Fig. 4 Cross sectional view of a typical dissolved air flotation unit with surface skimmer

tiny bubbles with supplied surfactants. However, producing small bubble in this method need not require a compressor and saturator which need in dispersed air flotation a promising technology for microalgae harvesting. Moreover, this technology requires less space and use less energy demand because of fewer mechanical components compare to dissolved air flotation (Nguyen et al. 2013). Mainly three types of surfactant were used widely as the collectors for microalgae cells removal such as nonionic X-100, cationic *N*-Cetyl-*N*-*N*-*N*-trimethylammonium bromide, and anionic sodium dodecylsulfate. These surfactants or collectors are used to prepare the surface of the microalgae cells for flotation by tiny air bubbles or to boost microalgae agglomeration. The mechanism behind is that during bubble formation, surfactant and air bubble changing the hydrophobicity of the microalage cells, which will affect the likelihood of microalgae-bubble attachment (Phoochinda et al. 2004) (Fig. 4).

3.3.3 Dispersed Ozone Flotation

Dispered ozone flotation or ozone flotation is more effective than air flotation for solid–liquid separation (Cheng et al. 2011). Microalgae dewatering and effluent treatment using ozone-induced flotation was studied by many authors and reviewed by Show et al. (2013). It was reported that that ozone gas stimulated microalgae cells to folate in the medium by altering or adjusting the microalgae cell wall surface that liberating particular cell wall surface active agents or biomolecules such as polysaccharides and proteins from microalgae cells. In a variation of dispersed flotation, ozone bubbles are injected that has been shown effective in the harvest of *Chlorella vulgaris* ESP-6 and *S. obliquus* FSP 3 (Cheng et al. 2010). In both their experiments, they studied at small scale, and it is unresolved how the oxidative properties of ozone would influence microalgae cells to float. However they predicted that, proteins released from the microalgae cells due to the ozonation perhaps helped the froth formation and cell adhesion to form top forth layer. The harvesting rate of microalgae, hydrophobicity and surface charge of microalgal cells, the content of algogenic organic matter (AOM) in polysaccharides and proteins

released from microalgae cells need determine and optimize during ozonation process to achieve desired harvesting rate. Cell wall proteins released from microalgae are strongly bound AOM and are most important to alter the hydrophobicity of bubble surfaces for easy attachment with microalgae cells which can form a top froth layer of floating microalgae for accumulation.

3.3.4 Foam Flotation

Foam flotation technique is similar to dissolved air flotation where bubbles generated by air compressor is required along with foam that contains cationic surfactant such as cetyl trimethylammonium bromide (CTAB) (Coward et al. 2013, 2014). There are two possible mechanisms of foam flotation. According to first hypothesis, the supplied cationic surfactant ions adsorb onto the microalgae cells and turn the cell hydrophobic that make cells available for bubble attachment at culture medium. The second hypothesis predicted that cationic surfactants alter the surface properties of the bubble by forming a positive charge to attach the negatively charged microalgae cells for floatation with foam for easy harvest. More study is required to confirm the hypothesis and understand the mechanism. Harvesting of *Chlorella* sp. has been done effectively by foam flotation during growth phase.

3.3.5 Electrolytic Flotation

Electric flotation for microalgae harvesting is a technology which can be use in small scale but this technology is not appropriate for large-scale- or open-pond culture system. Electric field-driven water hydrolysis generates hydrogen at the cathode and oxygen at the anode and these rising bubbles form in the medium that can attach microalgae cells and carries them to the surface of the medium to accelerate harvesting (Backhurst and Matis 1981; Mollah et al. 2004). The benefit of this method is that, this method is environmentally friendly as there is no need for the addition of chemicals to influence harvesting process. Recent year, (2017) one group performed a pilot project study with stainless steel as the cathode and carbon as the anode for microalgae harvesting using this method and achieved promising results for large-scale harvesting (Luo et al. 2017).

3.4 Centrifugation

Till now centrifugation is most commonly used method for microalgae harvest from small to large scale. Centrifugation is a mechanical process which involves to use

centrifugal force for separation of solid–liquids mixtures. Nowadays most of the commercial microalgae production units are harvesting biomass by centrifugation, even though it is very expensive. According to various reports, centrifugation is most effective in harvesting technology for microalgae in terms of recovery and minimizing water content with recovery rate over 90% within 2–5 min (Chen et al. 2015). Centrifugation requires high capital investment, energy and running cost which is only economical for high value products but not economical for low value product development such as biofuel from microalgae (Dassey and Theegala 2013). The technical and economic analysis on microalgae for biofuels showed that the investment costs for the centrifuges contributed up to 30 % of the total investment for this industry. The high gravitation and shear forces involved in the process may damage the cell structure and resulted the unwanted release of desired product which can be runoff with medium (Pragya et al. 2013). Various centrifugation devises are available in the market with different size and capacity such as hydrocyclone, tubular centrifuge, solid-bowl decanter centrifuge, nozzle-type centrifuge, solids-ejecting disc centrifuge, etc., and most of them were examined for microalgae separation from diluted culture medium. Some of them were proved as very efficient as one-step separation process, while others were found inefficient those required thickened algae slurry to obtained desired level of concentrated biomass (Show et al. 2013). Below photos show two different types of centrifuge devise frequently used in the production station of Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences (Fig. 5).

Fig. 5 Disk centrifuge (left), Tube centrifuge (right). Photos are taken from microalgae culture facilities of Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, China

3.5 Flocculation

Flocculation method is the most modern and economical method for microalgae calls concentration before final harvesting by centrifugation, to minimize the cost of huge water drain-off. Microalgae harvesting by flocculation is mainly divided into chemical flocculation, physical flocculation, and bio-based flocculation. Flocculation can also occur spontaneously when pH in the solution is increased to a certain high level which is termed as auto-flocculation (Brady et al. 2014). This section provides detail on various flocculation technologies for microalgae harvesting with critical discussions on their advantages and drawbacks.

3.5.1 Physical Flocculation

Harvesting of microalgae biomass via physical flocculation is a widely used method that can escape unwanted contamination induced by chemical flocculants. Various studies on physical flocculation reported that it can be achieved in various ways such as ultrasound waves, magnetic separation, electro-flocculation, etc. Application of ultrasound method to harvest microalgae cells has been studied by Bosma et al. (2003) and Heng et al. (2009). Although microalgae harvesting through ultrasound method works well in the laboratory scales, it is difficult to optimize and apply on commercial scale of harvesting. It is an energy-intensive process. When ultrasound was applied for the harvesting *Monodus subterraneus*, ultrasonic irradiation-coagulation exhibited and satisfactory recovery of algae biomass was achieved at operating parameters (40 kHz, 60 W, and 15 s) with an energy consumption of 345 kW d^{-1} (Heng et al. 2009). Microalgae cells density in culture, biomass flow rate, and rate of harvest and biomass flows during sonication, and conditions of sonication provide significant effect on the concentration factor during ultrasound method of microalgae harvesting. Nowadays, scientists are working on various nanoparticles for harvesting and oil extraction from microalgae; several studies have already been explored by using magnetic nanoparticles to harvest microalgae. It was claimed that, in this method, magnetic nanoparticles such as Fe_3O_4 patch to the cell surface of microalgae and prompt flocculation efficiency in the presence of magnetic field. The efficiency of Fe_3O_4 nanoparticles binding to cell surface depends of microalgae species and gives different results when applied on different species. (e.g., so far, *Chlamydomonas reinhardtii*, *Chlorella* sp., and *P. tricornutum* were harvested by Fe_3O_4 nanoparticles assistance when those were coated by canonic polymers or silica (Cerff et al. 2012; Lee et al. 2013; Lim et al. 2012). The benefit of using magnetic nanoparticles in harvesting microalgal cells is the viability of efficient reuse of the nanoparticles without pH adjustment. The disadvantage of this technology is the high price of magnetic nanoparticles and the requirement of special equipment and expert for recycling the nanoparticles for further use (Xu et al. 2011). Another innovative physical flocculation technology for harvesting of microalgae is electro-flocculation. This technology seems more

cost-effective and feasible to scale-up. In this harvesting method negative charged microalgal cells move to anode and loose the cell surface charge which facilitates the formation of microalgal aggregates or flocs. Then the bubbles produced at the anode upswing the microalgal aggregates or flocs to the upper surface for swept off easily. So far, aluminum electrodes showed preferable microalgae harvesting efficiency and required lower energy input (0.3–2 kWh kg^{-1}) during harvesting of *Nannochloris oculata* KMMCC-16 (Kim et al. 2012) and *P. tricornutum* (Vandamme et al. 2011). In addition another report, Fe^{2+} from steel electrodes can benefit electro-coagulation for harvesting of *Chlorococcum* sp. and *Tetraselmis* sp. at energy consumption of 0.31 kWh kg^{-1} and 0.54 kWh kg^{-1}, respectively (Uduman et al. 2011).

3.5.2 Chemical Flocculation

A wide range of organic and inorganic salts are used to flocculate microalgae for long time. It is well known that most of the microalgae cells have a negatively charged cell surface which confirms the cells stability in aqueous medium. In metallic salt-induced flocculation, a high dosage of costly flocculant and an acidic pH are required to achieve a satisfactory result and most researchers claim that this not economical and not environment friendly. In general, chemical flocculation of microalgae can be achieved by three main types of flocculants, namely, inorganic flocculants (including metal salts, ammonia, etc.), inorganic polymers, and organic polymers. Extensive studies have been done in this area and lot of literature shows successful applications of chemical flocculation in harvesting the cells of various microalgae species, such as *Chlorella* sp. (Papazi et al. 2009), *N. salina* (Rwehumbiza et al. 2012), *Neochloris* sp. (Beach et al. 2012), *Nannochloropsis* sp. (Rwehumbiza et al. 2012), *Scenedesmus* sp. (Chen et al. 2013) and *Phaeodactylum* sp. (Zheng et al. 2012).

Three common mechanisms may contribute to the chemical flocculation, namely, (I) charge neutralization and electrostatic patch, (II) bridging, and (III) sweeping. The charge neutralization and electrostatic patch process is a phenomenon that positive charged ions (e.g., Al^{3+} and Fe^{3+}), polymers (e.g., chitosan), or colloids (e.g., $Al(OH)_3$ and $Fe(OH)_3$) can strongly adsorb on the negative charged surface of microalgae cells or particles and even reverse the charge surfaces, which results in the collapse of electrostatic suspension and positive–negative attraction between the cells or particles, and then flocculation or aggregation will subsequently occur (Hjorth and Jorgensen 2012). Bridging is the process in which more than one cell or particle binds to the segments of polymers or colloids to form bridges among microalgae cells that causing cell aggregation and inducing flocculation (Biggs et al. 2000). In sweeping flocculation, cells or particles are entrapped by a massive aggregation which subsequently inducing the flocculation (Vandamme et al. 2013b).

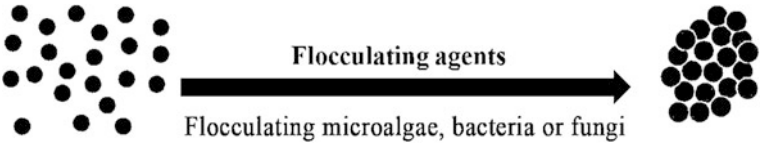

Fig. 6 Concept of bioflocculation of microalgae

3.5.3 Bioflocculation

Bioflocculation is the most recent innovative and economical approach of microalgae harvesting. Bioflocculation refers to the use of yeast, bacteria, fungi, or other algae to form or induce flocculation of the target freely suspended microalgae. This type of flocculation is usually caused by secreted biopolymers or extracellular polysaccharides (EPS) or proteins from flocculating organisms. Bioflocculation can be achieved in several ways. Several fascinated and economical approaches of microalgae harvesting using bioflocculation methods have been explored and reported in recent years (Alam et al. 2016b) (Fig. 6).

Bacterial communities have a significant role in the process of microalgae aggregation in the natural aquatic systems; thus coculture of microalgae and bacteria has been a practice for a long period of time for wastewater treatment of control of algae bloom. Nowadays, scientist use bacteria to flocculate freely suspended microalgae for harvesting. Different bacteria may produce and secrete different kinds of bioflocculants that enhanced microalgae sedimentation. In a study, a protein-based bioflocculant produced by *Solibacilus silvestris* was reported to be effective in flocculation of freely suspended marine microalgae *Nannochloropsis oceanica* without addition of chemical flocculant and achieved improved flocculation efficiency. This reported bioflocculant is a significant improvement from earlier published reports, since it can be recycled, avoiding secondary contamination, and resulted in enhanced flocculation efficiency there by reducing the overall cost of microalgae cell harvesting process (Wan et al. 2013). Recent years, several filamentous fungi species are found to be useful for biological flocculation of microalgae under controlled operation condition (More et al. 2010; Zhou et al. 2013). The mixture or coculture of non-filamentous microalgae cells with filamentous fungi form cell pellets and can induce bioflocculation for easier harvest of microalgae. The mechanism is not well studied yet but it was predicted that fungal hyphae and mycelia have polysaccharide with active sites which may enable their surface capability of bioadsorption of the microalage and also enable the fungal cells to be positive charged to attach negative charged microalgae cells (Zhang and Hu 2012). So far a few number of filamentous fungi such as *Mucor circinelloides*, *Penicillium expansum,* and *Rhizopus oryzae* were reported to form pellets with 2–5 mm of diameter and accelerated microalgae harvesting. It was reported that mixed cultures of *C. vulgaris* and *C. echinulata* (algae to fungi ratio of 2:1) can achieve 99% of the microalgal biomass recovery at the end of 2 days of coculture.

Another recent innovative idea is to harvest freely flocculated microalgae cells with coculture of self-flocculating microalgae. Thus strategy is very much economic and environmental friendly as no other associated cost involved for purification of chemical or unwanted microbes and biomass harvested by this process can be used in wide range from high value product such as chemical or food additives to low value product such as biofuel (Alam et al. 2016b). So far, only few self-flocculating algae have been reported, such as *C. vulgaris* JSC-7, *Ankistrodesmus falcatus*, *Scenedesmus obliquus* and have been used to enhanced flocculation of non-flocculating microalgae cells (Alam et al. 2014; Guo et al. 2013; Salim et al. 2011). In last decades, significant effort has been put on the identification of potential biofuel production strains with higher content of lipid or TGA; those are capable to grow in mass culture but identification of self-flocculate microalgae to improve the economics of harvesting has not been studied widely. Moreover, most of the study on self-flocculation of microalgae has been done only in lab-scale- and pilot-scale study is badly needed. For example, the coculture of self-flocculating strain *C. vulgaris* JSC-7 with the non-flocculating algal strains, *C. vulgaris* CNW11 or *S. obliquus* FSP, increased flocculation of the non-flocculating strains to nearly the same rate as showed by JSC-7 alone. For CNW11 cocultured with JSC-7, the harvesting efficiency increased three times (from 25.6% to 68.3 and 34.8 at 1:2 and 1:5 dilutions, respectively). When cocultured with *S. obliquus*, the efficiency also enhanced from 28.1 to 62.7% when diluted 1:2 at ratio and to 41.2% when diluted 1:5 at ratio with JSC-7 (Alam et al. 2014). In the same reports, they studied the mechanism of flocculation in this mixture and reported that a polysaccharide-based flocculating agent extracted from the cell wall of flocculating microalgae *C. vulgaris* JSC-7 have showed strong capability in flocculating the CNW11 strain (>85% efficiency within 1 h) with a concentration as low as 0.5 mg/L (Alam et al. 2014). The similar dose of polysaccharides extracted from the cell wall of *S. obliquus* AS-6-1 can achieve a flocculation efficiency of 80% in the same strains (Guo et al. 2013). Studies suggested that both bridging and patching mechanisms involved in bioflocculation process of microalgae. When a large network of microalgal cells is formed between algae cell to cell, the mechanism involved is bridging, and while the cells seem more closely attached, the mechanism involved may be patching by the EPS excreted from flocculating microalgae, details in (Fig. 7).

Genetic modification towards developing new flocculating strains with high lipid content may also be a future capable way to harvest microalga for biofuel production. Algal strains could be are engineered with gene transfer for triggers flocculation (Díaz-Santos et al. 2015; Alam et al. 2012). Mechanism of yeast flocculation and its genetic modification aspect has been intensively studied to facilitate biomass recovery in the brewing and ethanol industry. The expression of FL0 gene from *Saccharomyces cerevisiae* in fresh water microalgae has been developed and flocculating mutants were obtained. Transgenic microalga *C. vulgaris* ZRA01 containing flocculating characteristic showed a great flocculating activity then natural self-flocculating microalgae and freely suspended species

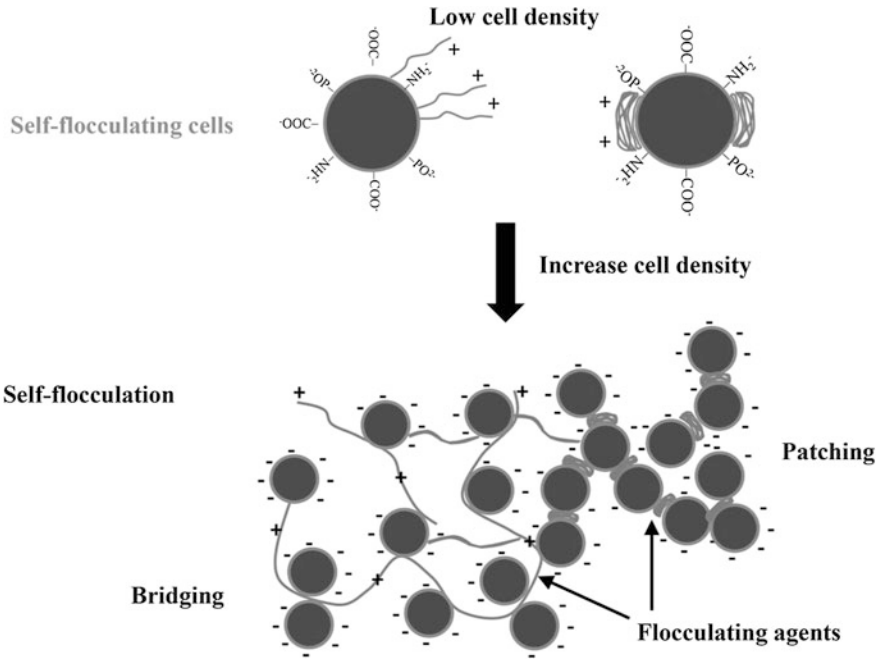

Fig. 7 The proposed mechanism for microalgal bioflocculation

(Alam et al. 2012). The FL05 gene from *Saccharomyces bayanus* has been transferred into *Chlamydomonas reinhardtii* and transformants exhibited a 2–3.5-fold increase in self-flocculation abilities compared to the untransformed control strain (Díaz-Santos et al. 2015).

4 Economics of Flocculation Based Microalgae Harvesting

Mass production and application of microalgae biomass is still economically not viable compare to agricultural biomass for fuel development due to lack of cost-effective cultivation and harvesting methods of microalgae. Extremely high volumes of water need to be processed during harvesting of macroalgae biomass. This leads to high electricity and heat consumption which compromise significantly big amount of energy and capital investment. Within the last decade, considerable advances have been made to explore new technologies for harvesting of microalgae but there is only a limited amount of information have been gather by comparing the economical aspect of those techniques (Allnutt and Kessler 2015; Jonker and Faaij 2013). Most of the reported techno-economic analyses focused on production cost only. Generally flocculation is part of a two-step harvesting process in which flocculation is used to preconcentrate the algal biomass to remove maximum water

from culture system then final dewatering done by physical method. The more water can be removed during the first flocculation steps, the lower the cost will be for the second mechanical dewatering step.

Harvesting microalgae using physical and chemical flocculation is still expensive for commercial application of biofuel production. The cost of autoflocculation at high pH slightly is less expensive than centrifugation. However, chemical flocculation technologies resulted in contamination of the harvested biomass with chemicals or minerals which may hinder for some valuable product development and also need recycling of culture medium for reuse which requires additional indirect cost (Wan et al. 2015). The most promising low-cost flocculation method is bioflocculation. Bioflocculation by addition of flocculating microalgae or other organisms involve lower cost compared with centrifugation directly, which is currently considered most promising and innovative method for harvesting microalgae in laboratory scale (Alam et al. 2016b). However more study is needed to optimize this technology in pilot scale to facilitate commercial application. Most bioflocculation methods till now studied only single parameter such as removal efficiency, thus more study is necessary to explore the key parameter such as settling velocity and the concentration factor of bioflocculation processes. Genetic modification may be a future promising approach to commercial harvesting microalgae cells. But we need to consider the impact of GM and should study the impact on environment before release modified strain. Bioflocculation especially microalgae assistant flocculation is a significant and innovative improvement over other harvesting methods which need to commercialize. In this method, the microalgal cells are not damage, maintain their integrity and associated investment cost is negligible. At industrial scales, culture media could be reused to minimize the cost of nutrients and the demand for water.

5 Conclusion

To produce biofuel from microalgae is of course technically possible but it is essential to resolve many of bottlenecks from algal culture to fuel production allied with economical, engineering, and biological aspects to reduce the production costs as microalgae-derived biofuel is relatively expensive compared with petroleum fuels due to the lack of economically feasible technology. Bioflocculation or genetically modified flocculating microalgae is one of the great options to achieve cost-competitive production systems to displace current liquid fuels. Based on the review findings, although lots of lab-scale researches have fostered to achieve economic harvesting method using various harvesting methods, but significant large scale-demonstration is require developing commercial scale of microalgae harvesting by bioflocculation. Cross-pollination among research institute (integration of biology and engineering), expertise and microalgae industries will thus require achieving this goal by more researching in lab-scale- and large-scale basis.

Acknowledgements We thank NSFC International (Regional) Cooperation and Exchange Project for Young Scientist (Project No. 21650110457), National Natural Science Foundation for research team of Guangdong, China (Grant No. 2016A030312007), and National Natural Science Foundation of China (NSFC) (Grant No. 51476177 and 31070441) for funding support.

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Microalgae-Based Biorefineries as a Promising Approach to Biofuel Production

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Abstract Microalgae are photosynthetic microorganisms that are capable of converting carbon dioxide, nutrients, and solar energy into biomass and oxygen. In addition, microalgae have high photosynthetic rates, do not require potable water and arable land for cultivation, and can use liquid and gaseous effluents as nutrients for growth. The biochemical composition of microalgae can be manipulated by changing the cultivation conditions and environmental stresses. Thus, these microorganisms can be induced to produce biomass that is rich in biocompounds of commercial importance. The microalgal biomass can be converted into biofuels and high value-added bioproducts. Thus, microalgae have potential uses as renewable raw materials and could provide promising materials for the development of biorefineries. In this context, this chapter examines microalgae within a biorefinery concept and describes the advantages of using microalgae, culture conditions, biocompounds from biomass and the potential for converting them into biofuel and bioproducts.

1 Introduction

The burning of fossil fuels is a major contributor to the increase in greenhouse gases (GHG) in the atmosphere and is directly linked to global warming. Fossil resources are not considered sustainable and are economically, socially, and environmentally questionable (Kamm et al. 2006). For these reasons, energy sources that are sustainable and environmentally friendly to society and the global economy are needed (Mabee et al. 2005).

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B.N. Tripathi and D. Kumar (eds.), *Prospects and Challenges in Algal Biotechnology*, DOI 10.1007/978-981-10-1950-0_4

With their potential to generate sustainable clean energy, microalgal biomass has favorable characteristics for producing biofuels and high value-added bioproducts. In the multiproduct paradigm, microalgae are part of a select group of raw materials that can be used in biorefineries (Subhadra 2010).

The process of obtaining bioproducts becomes costly when it is based on only one conversion technology. Conversion technologies, such as thermochemically and biochemically derived items, can be combined by using biorefineries to reduce the overall cost of the process, yielding greater flexibility during the production of bioproducts (Naik et al. 2010). Some studies have focused on the use of microalgae products for different applications (Lu et al. 2004; Mandal and Mallick 2009; Chen et al. 2011); however, most studies are focused on obtaining only a specific product from microalgal biomass. Thus, the other available and valuable components of microalgae are lost (Vanthoor-Koopmans et al. 2013).

The microalgal biomass has high concentrations of lipids, proteins, and carbohydrates, which can be used for different applications. When the full potential of the microalgal biomass constituents is exploited, many bioproducts can be obtained simultaneously and the market value is greater than the production costs (Wijffels and Barbosa 2010).

The great advantage of using microalgae as a raw material for biorefineries is that they do not compete with food crops, because they do not require arable land, in addition to their use of effluents as nutrients for their development. Microalgae grow faster than land plants and thus have a higher CO_2 fixation rate (Chen et al. 2011). In addition, the biochemical composition of microalgae can be manipulated by changing the growing conditions and environmental stresses. Thus, these microorganisms can be induced to produce high concentrations of commercially important biocompounds (Hu et al. 2008; Goiris et al. 2015).

Based on these previous studies, this review examines the use of microalgae in designing a biorefinery. The advantages of using microalgal biomass compared to other substrates, the use of effluents as nutrients, and the conversion of biomass into biofuels and high value-added bioproducts are described.

2 Refinery Versus Microalgae Biorefinery

The demand for actions to mitigate GHG emissions and the increased costs of obtaining oil coupled with instability in oil prices due to geopolitical factors have motivated the creation of alternatives based on renewable resources (Bennett and Pearson 2009; Cherubini 2010; Kokossis and Yang 2010). In this context, biorefineries offer the opportunity to obtain products with the same applicability as products obtained from petroleum, but they are generated from renewable raw materials (Ghatak 2011).

Biorefineries use integrated industrial processes, in which biomass is converted into a series of biochemical compounds, energy, and chemical products with high added value. Biorefineries are analogous to oil refineries, in which multiple fuels

and chemicals are produced from fossil fuel (Romero-García et al. 2014). Biorefineries and refineries involve processes in which raw materials are separated into different fractions. In a petrochemical refinery, the crude oil is separated and "refined" into products such as fuel, the building blocks for chemicals and materials for road construction. However, the raw material used in a biorefinery (biomass) is separated into fractions that may consist of sugars, cellulosic components, proteins, and lignin, which will be "refined" later and then used for biofuel and chemical production, used as food ingredients, or burned to generate energy (Cherubini et al. 2009).

Bennett and Pearson (2009) argue that this analogy may be questionable. The fractions obtained from distillation and oil cracking are distinct, and they are manufactured separately during the production of gasoline, diesel, turbine oil or olefins. The initial steps of biomass processing will produce fractions, such as cellulose or synthesis gas, to serve as raw materials for fuels and biochemical compounds. Therefore, there is direct competition among biomass uses. According to Kamm et al. (2006), traditional refineries employ oil as the raw material that primarily supplies transport and energy fuels, and only a relatively small fraction is directed to chemistry. In a biorefinery, a relatively greater amount may be directed to biochemistry and the production of biocompounds; however, bio-based products can only compete with petrochemicals when the biomass resources are optimally processed by biorefinery systems.

By producing various bioproducts, biorefineries can be used to explore the full potential of biomass, adding value, increasing profitability, and reducing energy demand and GHG emissions. The ability to obtain different bioproducts also lowers production dependence on only one product, thereby improving the sustainability of the rational use of biomass, reducing the competition between uses for food or fuel (Ghatak 2011). In the context of microbial biorefineries, microalgae are intensively studied because they can consolidate the production of biofuels and high value-added bioproducts (Silva et al. 2014). Figure 1 shows a schematic diagram of a microalgae-based biorefinery.

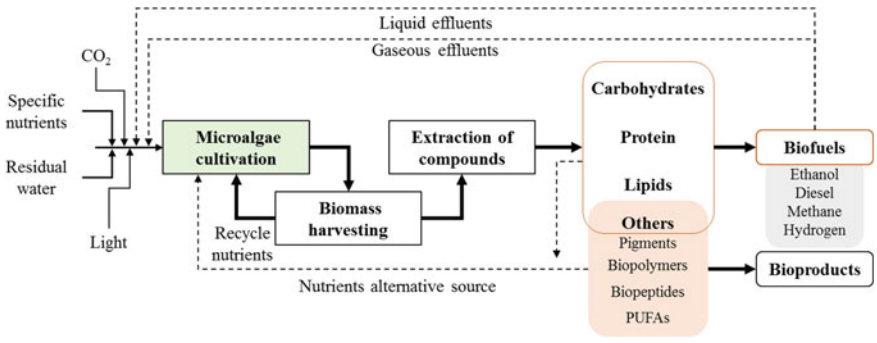

Fig. 1 Schematic diagram of a microalgae-based biorefinery

Table 1 Concentrations of the main macromolecules from microalgae on a dry basis (%, w w^{-1})

Microalga	Proteins	Carbohydrates	Lipids	Reference
Anabaena cylindrica	43–56	25–30	4–7	Demirbas (2010)
Chlamydomonas reinhardtii	64–66	20–25	13	Mahdy et al. (2014)
Chlorella vulgaris	61–66	19	18	Mahdy et al. (2014)
Dunaliella salina	28–30	16	7–8	Díaz-Palma et al. (2012)
Scenedesmus obliquus	42	19	32.5	Wu and Miao (2014)
Spirulina sp.	60–65	14–22	5–10	Rosa et al. (2015)
Tetraselmis maculate	52	15	3	Demirbas (2010)

The cultivation of microalgae is an efficient option for the bioremediation of wastewater through the recovery of high levels of nitrogen, inorganic phosphorus, and heavy metals from effluents. Furthermore, microalgae reduce CO_2 emissions from gaseous effluents and the atmosphere, converting carbon and solar energy into biomass through photosynthesis (Schneider et al. 2012). The composition of microalgal biomass may vary across species (Table 1) and cultivation conditions, and it primarily consists of lipids, carbohydrates, and proteins (Zhu 2015).

Microalgal biomass has the potential to provide renewable energy in forms such as biodiesel, bioethanol, biohydrogen, and biogas. Moreover, these photosynthetic microorganisms also have the capacity to synthesize bioactive molecules, such as carotenoids, polyunsaturated fatty acids, biopolymers, antioxidants, anti-inflammatory compounds and other organic compounds that can be used in human and animal nutrition, cosmetics, biomaterials, nanostructures, and drugs (Marques et al. 2011).

The development of the biorefinery concept may enable biofuel generation through the production of co-products to promote the better use of all the biomass components (Trivedi et al. 2015). Olguín (2012) reported a proposed biorefinery for two combined purposes, for cultivating oleaginous microalgae with wastewater and cultivating *Arthrospira* with effluent from the anaerobic digestion of animal waste and seawater, both of which are used for the production of biofuels and products with high added value. At this phase, oleaginous microalgae growth is proposed for the production of three biofuels, biodiesel, biogas and biohydrogen. *Arthrospira* cultivation is proposed for biogas production and high-value products, such as fish food, phycocyanin, and PUFAs.

2.1 Microalgae Cultivation Within the Biorefinery Concept

Microalgae are photosynthetic microorganisms that belong to a large and diverse group including both unicellular and multicellular organisms and both prokaryotes (cyanobacteria) and eukaryotes (Li et al. 2008). These microorganisms have high cell growth rates, with a division every one to two days under favorable growing

conditions (Williams and Laurens 2010). Compared to terrestrial plants, the cell growth of microalgae can be 100 times faster (Lam et al. 2012).

The microalgae habitat can be aquatic (Becker 2004) or terrestrial, with over 40,000 species already cataloged. Among the major groups of microalgae, Cyanophyceae (blue algae), Chlorophyceae (green algae), Bacillariophyceae (diatoms), and Chrysophyceae (golden algae) are often cited when describing desirable characteristics for the efficient and economical combination of CO_2 fixation, wastewater treatment and the synthesis of lipids for biofuel production (Kumar et al. 2010).

Due to the commercial potential of microalgal biomass, the research and development of technologies for the production and harvest of microalgae are being conducted by several private companies and academic institutions. Earthrise Nutritionals, which is located in California (USA), and Cyanotech Corporation, in Hawaii (USA), are two companies involved in the production of *Spirulina* using open raceway ponds. Sapphire Energy has an advanced facility for producing fuel from algae. This company has several patents on processes such as the heat treatment of crude seaweed, a process for the recovery of oily biomass compounds to produce biofuels from prokaryotes and eukaryotes and the induction of flocculating photosynthetic organisms (Christenson and Sims 2011). Seambiotic is an Israeli company that grows microalgae in outdoor reactors near power plants. Concentrated CO_2 from the flue gas is fed to the cultures (Weiss 2008). General Atomics Company has several patents related to algae cultivation (Christenson and Sims 2011).

Many companies market nutraceuticals that are developed using microalgal biomass. Natureza Beta Technologies in Israel provides a range of microalgae-based products such as cosmetics and food supplements. Tablets, chips, creams, liquid extract, and *Spirulina* powder are marketed in Yangon (Myanmar) (Spolaore et al. 2006).

In Brazil, the Laboratory of Biochemical Engineering (LEB) at the Federal University of Rio Grande (FURG) has been developing research on microalgae cultivation since 1996. Since then, the photobioreactor settings and cultivation modes (Reichert et al. 2006), medium renewal rate and blend concentrations (Reichert et al. 2006; Moreira et al. 2016), agitation (Henrard et al. 2014), light intensity, temperature, nutrient composition, and use of alternative substrates in the supplementation of the culture medium (Borges et al. 2013) have been studied. Native strains such as the cyanobacterium *Spirulina* (Morais et al. 2008) and microalgae with potential for CO_2 biofixation (Morais and Costa 2007a; Radmann et al. 2011) were also isolated (Morais and Costa 2007b).

Since 1998, LEB has been developing a study project on *Spirulina* cultivation on a pilot scale at the edge of Mangueira Lagoon (33° 30′13″ S, 53° 08′ 59″ W) (Morais et al. 2009). With this search, a pilot version of a *Spirulina* biomass production plant was designed and implemented in the city of Santa Vitória do Palmar (RS) (Fig. 2a), with a production capacity of 70 kg month^{-1}. The biomass

produced there is used to supplement local school lunches and for developing foods such as instant noodles, powdered cake mix, cookies, pudding, powdered chocolate, instant soup, powdered gelatin, cereal bars, and foods intended for practitioners of physical activity (Costa and Morais 2011).

The biomass produced by microalgae cultivation in several studies was evaluated for the production of fatty acids (Colla et al. 2004), biopolymer extraction (Martins et al. 2014), nanofibers (Morais et al. 2015a), the development of protein hydrolysates (Lisboa et al. 2014), and nanoemulsions. The potential for developing biofuels from compounds that could be extracted from the biomass, such as bioethanol (Margarites and Costa 2014), biodiesel, and biogas, was verified.

Among the LEB projects, the one focused on CO_2 biofixation by microalgae began in 2005 with an agreement with Eletrobrás and the Electric Power Thermal Generation Company (CGTEE). As the research advanced, a pilot plant for CO_2 biofixation by microalgae was designed and built in the Presidente Médici Thermal Power Plant in the city of Candiota (RS) (Fig. 2b). The plant has an area of 6,000 m^2, with 70 m^2 of laboratories, and three raceway-type bioreactors. Two of the bioreactors have a working volume of 18 m^3 each, and one has a volume of 1 m^3 for growth and inoculum maintenance (Costa et al. 2015).

Based on the signed agreement, several studies related to CO_2 biofixation by microalgae have been developed. These studies focus on the development and use of different photobioreactors, integrated chemical, and biological CO_2 fixation (Rosa et al. 2015), and the use of CO_2 and coal-combustion gas in the cultivation of microalgae species (Morais and Costa 2007a, 2007c; Radmann et al. 2011) as well as the evaluation of compounds in the biomass (Radmann and Costa 2008). In this sense, research on microalgae cultivation and the production and evaluation of the microalgal biomass potential were investigated to enable the sustainable development of a microalgae biorefinery.

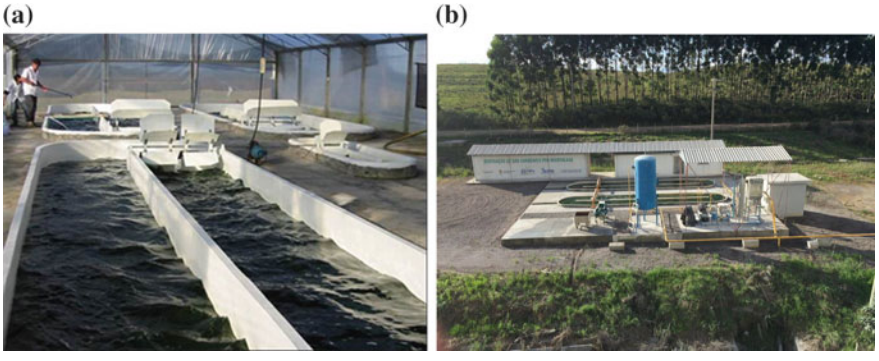

Fig. 2 **a** Pilot plant for producing *Spirulina* biomass in Santa Vitória do Palmar-RS, Brazil, and **b** a pilot plant for microalgal CO_2 biofixation in Presidente Médici Thermal Power Plant, Candiota-RS, Brazil

2.2 Microalgal Biomass Compared to Other Substrates

The term "biomass" refers to the raw materials that are used in biorefineries, which are renewable and carbon-based and come from four different sectors. Those sectors are agriculture (cultures and residues); forestry (growing trees); industrial (process residues) and urban (household and commercial residues); and aquaculture (microalgae and macroalgae) (Cherubini 2010). The term "biomass" may also be categorized into sections such as biomass originating from cultures or residues. The cultures include raw materials with sugarcane, grains of different cultivars, microalgae and macroalgae, and plant oils such as palm and lignocellulosic materials including wood and grass. Residues can be classified as being of agricultural, forestry, industrial, and municipal origin (Naik et al. 2010).

Agricultural residues include straw, bark, and mulch from crops such as rice, wheat, and corn, while forest residues may contain sawdust, shavings, and wood shavings. Wastewater and solid waste materials are classified as municipal sources and may come from households and commercial establishments (Naik et al. 2010).

As reported by Balat et al. (2008) and Naik et al. (2010), crops such as sugar cane, sugar beets, maize, sorghum, wheat, and barley can be used to produce bioethanol. The world's largest bioethanol producers are the United States and Brazil, and they use corn and sugarcane as raw materials, respectively (Sarkar et al. 2012). The disadvantages associated with the use of these raw materials are their influence on the food supply and their use of large amounts of land (Harun et al. 2010b).

Vegetable oils and animals fats are extracted or pressed to obtain crude oil or fat that can be used in biodiesel production (Ma and Hanna 1999). Conventional biodiesel primarily comes from soybean and vegetable oils, palm oil, sunflower oil, and rapeseed oil as well as restaurant waste oil. The production cost of biodiesel consists of two primary components, namely, the cost of raw materials (fats and oil) and the cost of processing (Huang et al. 2010). The sustainable and economic production of biodiesel from vegetable oils has been challenging due to their significant land use and sustainability issues. The use of residues such as cooking oil or animal fat is an effective method of recycling residues; however, refining and hydrogenation are needed to make biodiesel usable (Ma and Hanna 1999).

Agricultural and forest residues from the post-harvest industrial processing of food cultures can generate huge amounts of carbohydrates containing lignocellulosic residues (Huber and Corma 2007). Lignocellulosic biomass is made up of three primary building blocks, namely, cellulose, hemicellulose, and lignin. This raw material is considered the second-generation and can be used in the production of biofuels and chemicals through various conversion technologies (Naik et al. 2010). The biochemical and thermochemical routes (Cherubini 2010) may use lignocellulosic biomass for biofuel production. However, studies show that the major obstacle to the widespread utilization of this important resource is the absence of economically feasible technologies for overcoming the impediments of converting lignocellulosic materials to biofuel (Yousuf 2012). Therefore, it remains

debatable as to whether the use of second-generation biofuels could help to meet the global demand for fuels in the transportation sector by 2030 (Sims et al. 2008).

In this context, microalgae have attracted attention in recent years as a renewable source of biomass with advantages over traditional energy cultures (Demirbas 2011). The microalgal biomass is considered a rich source of phytochemicals that can be used in food and feed, aquaculture, pharmaceuticals, dietary supplements, cosmetics, and health products as well as for producing biofuels and other bioproducts (Pulz and Gross 2004; Spolaore et al. 2006; Brennan and Owende 2010).

In oleaginous energy cultures, the production cycle lasts for 3 months to 3 years; however, for microalgae, biomass harvesting may begin from 3 to 5 days and can be extracted daily. These photosynthetic microorganisms are produced throughout the year, unlike most vegetable crops, which are seasonal. With support for their high photosynthetic efficiency, algae have higher growth rates and biomass productivity compared to terrestrial plants as well as the ability to fix greater amounts of CO_2. Microalgae do not require potable water and fertilizer, given that they are able to absorb nutrients from wastewaters and gas effluents. Finally, due to the low cultivation demands of these microorganisms, these cultures can be implemented on degraded land, in the desert and even on offshore structures, thereby eliminating competition with the food sector (Demirbas 2011).

Microalgae have a favorable composition for use in biorefineries, with ash contents between 4 and 10% w w^{-1} and a low lignin concentrations in the cell wall. Therefore, at least 90% w w^{-1} of microalgal biomass can be converted into marketable bioproducts. The productivity of microalgae biomass (60–100 ton ha^{-1} a^{-1}) can be higher compared to other substrates used in biorefineries, such sugarcane (60 ton ha^{-1} a^{-1}), soybeans (3 ton ha^{-1} a^{-1}), and wood (pine) (3 ton ha^{-1} a^{-1}); however, applications are required at a larger scale (Gerardo et al. 2014). As a result of the features discussed here, microalgal biomass can be considered an excellent raw material for use in biorefineries to produce biofuels and high value-added bioproducts (Zhu 2015).

2.3 Liquid and Gaseous Effluents as a Source of Nutrients

2.3.1 Liquid Effluents

Several investigators have investigated the cultivation of microalgae with the aim of defining the ideal conditions for maximizing growth rates and composition of microalgal biomass; however, microalgal cultures are still dependent on (i) the cost, (ii) the CO_2 capture sources, (iii) the target product, and (iv) the nutrient sources (Cheah et al. 2015).

In addition to carbon, microalgae can assimilate nitrogen and phosphorus from the culture medium to maintain efficient metabolic activity. Nitrogen is an important element and is a necessary nutrient of microalgae, as the primary constituent of nucleic acids and proteins (Green and Durnford 1996). Ammonium is the primary

nitrogen source for microalgae (Razzak et al. 2013). Furthermore, phosphorus is necessary for photosynthesis, metabolism, and the formation of DNA, ATP, and the cell membrane. Phosphorus is available in the medium as phosphate and is usually supplied in excess, because it is not readily bioavailable. Other inorganic salts and trace elements such as metals and vitamins are normally added to the medium to maintain photosynthetic activity (Cheah et al. 2015). Nutrients represent approximately 10% of the costs of cultivation (0.44 € $kg_{dry\ biomass}^{-1}$) (Norsker et al. 2011).

Microalgae are known for their high nutrient-removal efficiency because they require large amounts of nitrogen and phosphorous to synthesize proteins, nucleic acids, and phospholipids, which represent 40–60% w w^{-1} of the cell's dry weight (Silva-Benavides and Torzillo 2012). Nutrients for the cultivation of microalgae (primarily nitrogen and phosphorus) may be obtained from liquid effluents, such as wastewater. Depending on the source, effluents can be classified as municipal, domestic, or liquid waste from agriculture and industrial products (Chiu et al. 2015).

The use of wastewaters from primary or secondary wastewater treatment is an economically and environmentally promising solution because they contain significant amounts of these nutrients. In wastewater treatment plants, these nutrients must be removed because they contribute to eutrophication. This removal may increase the total energy consumption of treatment by 60–80% (Lam and Lee 2012).

The full process of nitrogen and phosphorus removal from tertiary wastewater treatment is almost four times more expensive than primary treatment (Noüe et al. 1992). Accordingly, the cultivation of microalgae offers a promising solution for tertiary treatment, because these microorganisms use inorganic nitrogen and phosphorus for their growth (Oswald 1988; Tam and Wong 1996). Microalgae can also remove heavy metals and toxic organic compounds, preventing secondary pollution (Chiu et al. 2015). In this context, microalgae can act as important agents in the bioremediation of wastewater (Zhou et al. 2014), with advantages over conventional systems including reduced costs through reduced energy consumption and low initial capital and operating costs (Wong and Tam 1998).

The concentrations of total nitrogen (TN) and total phosphorus (TP) following the secondary treatment of wastewater are relatively low (TN: approximately 15–90 mg L^{-1}, TP: approximately 5–20 mg L^{-1}). The TN and TP concentrations in wastewater from cattle breeding and agriculture are usually 185–3213 mg L^{-1} TN. For effluents such as manure from pig farms, chicken breeding facilities, and dairy farms, wastewater is digested anaerobically, and the resulting phosphorus concentration is approximately 30–987 mg L^{-1}. However, these types of wastewater contain extremely high nutrient concentrations and thus must be diluted before being used to cultivate microalgae (Chiu et al. 2015).

Gonçalves et al. (2014) evaluated the effects of light (36, 60, 120, and 180 μE m^{-2} s^{-1}), photoperiod (10:14, 14:10, and 24:0), CO_2 fixation and removal of nutrients (nitrogen and phosphorus) from the culture medium of four strains of microalgae (*Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina,* and *Microcystis aeruginosa*). The authors observed that all the microalgae showed high nitrogen-removal efficiencies, reaching 100% in the assay with the

highest brightness. Phosphorus removal increased with light irradiation and an increasing light-dark photoperiod ratio. The highest phosphorus removal efficiency (67.6% w w^{-1}) was attained by *C. vulgaris*.

2.3.2 Gaseous Effluents

The gas mixture generated by the combustion of fossil fuels is composed of CO_2, sulfur, and nitrogen oxides. These gases can be used as a source of nutrients for microalgae cultivation; however, they can also be toxic, reducing the pH of the culture medium and inhibiting growth if they are added directly and in an unstructured manner (Zhao et al. 2015).

Carbon is one of the major components of microalgae and is needed at high concentrations for cultivation. Microalgae are composed of approximately 50% w w^{-1} carbon. This nutrient may be provided by atmospheric CO_2 from industrial gases (flue gas) or various other sources (Becker 1994). According to Grima et al. (2003), the supply of CO_2 as a carbon source can represent up to 41.3% of the total cost of producing microalgal biomass. In this context, the use of waste gases from combustion containing from 5 to 15% v v^{-1} CO_2 can provide sufficient amounts of carbon to produce microalgae on a large scale (Kumar et al. 2010), adding value to the combustion processes.

Microalgae can efficiently fix CO_2 from the atmosphere and industrial combustion gases through photosynthesis. Their capture capacity is approximately ten times higher than that of land plants. These microorganisms may accumulate inorganic carbon in their cytoplasm at greater concentrations by several orders of magnitude compared to the outside of the cell, a phenomenon called CO_2 concentration (Kaplan and Reinhold 1999; Badger et al. 2006; Pires et al. 2012). As reported by Chisti (2007), 1.8 kg of CO_2 is required to generate 1.0 kg of biomass.

CO_2 fixation by microalgae is a promising method for the post-combustion capture and storage of carbon. Compared to other methods, the biological conversion of CO_2 by microalgae has several advantages, such as wide distribution, good adaptability to the environment and low operating costs. In addition, the microalgal biomass produced during this bioprocess can be used in the production of bioenergy as well as for extracting high value-added biomolecules, representing the additional benefits of the process (Zhao and Su 2014). These benefits expand on the integrated concept of a microalgae biorefinery (Zhu 2015).

The selection of appropriate strains for CO_2 fixation has a significant effect on cost competitiveness and process efficiency. The desirable characteristics of microalgae for high CO_2 fixation rates include high rates of growth and consumption of CO_2; a high tolerance of trace constituents in the flue gases, such as SO_x and NO_x; an ability to produce bioproducts; ease of harvest; characteristics associated with spontaneous sedimentation or flocculation; tolerance to high temperatures of the liquid medium to minimize cooling costs of flue gases; and the ability to be cultivated with wastewater treatment effluent (Brennan and Owende 2010).

The presence of SO_2 can inhibit microalgal growth. Some species of microalgae are capable of growing at high SO_2 concentrations; however, a longer acclimatization period is needed compared to cultures grown without SO_2. At increased SO_2 concentrations, the inhibitory effect is enhanced, resulting in a marked reduction in the carbon fixation and biomass productivity (Lee et al. 2000).

In flue gases, the NO_x levels may vary from hundreds to thousands of ppm, with more than 90–95% NO and 5–10% NO_2 (Yoshihara et al. 1996). When the SO_2 concentration is high (>400 ppm), the pH of the medium decreases, resulting in low biomass productivity; however, there is no evidence of a direct influence on microalgal growth by NO at concentrations of approximately 300 ppm because NO is absorbed by the culture medium and then converted into NO_2. It can thus be used as a nitrogen source (Kumar et al. 2010).

Several studies have been conducted over the years to examine the use of combustion gases in microalgae cultivation. Negoro and Shioji (1991) evaluated the effects of SO_x and NO_x on the growth of ten strains of halotolerant marine microalgae. With 15% v v^{-1} CO_2, the growth of *Nannochloris* sp. and *Nannochloropsis* sp. was not affected at 50 ppm SO_2; however, at 400 ppm, the pH was reduced and growth ceased after 20 h of cultivation. The same strains were tested at a high level of CO_2 and 300 ppm NO. In the absence of significant changes in the pH, both growth profiles were affected by the presence of these pollutants. *Nannochloropsis* sp. showed no cell growth, while *Nannochloris* sp. grew after a long latency period.

Morais and Costa (2008) evaluated the removal of GHG by *Spirulina* sp. and *Scenedesmus obliquus* in 2 L Erlenmeyer bioreactors, which were aerated with a synthetic gas mixture (6% v v^{-1} CO_2, 100 ppm NO, and compressed air). The authors report a maximum fixation of 22.3% for CO_2 and 27.1% for NO in cultivation with *S. obliquus*.

During the cultivation of *Chlorella* sp. MTF-7, (Chiu et al. 2011) found that the maximum biomass concentrations reached 1.67, 1.50 and 1.32 g L^{-1} in mixtures containing CO_2 at 2, 10 and 25% v v^{-1}, respectively, and 2.4 g L^{-1} using commercial combustion gas as a carbon source. Radmann et al. (2011) found that a synthetic flue gas injection containing 12% v v^{-1} CO_2, 60 ppm SO_2 and 100 ppm NO in *Spirulina* sp., *Chlorella vulgaris* and *Scenedesmus obliquus* cultures caused no growth inhibition, reaching maximum biomass yields of 80, 90, and 60 mg $L^{-1}d^{-1}$, respectively.

The liquid effluents of waste and gaseous waters from the combustion of fossil fuels can be used as nutrients for cultivating microalgae. The integration of liquid and gaseous effluent treatments by microalgae can not only contribute to sustainability in wastewater treatment (Rawat et al. 2011; Razzak et al. 2013), but also turn out to be a sustainable process for producing microalgal biomass within the biorefinery concept (Pires et al. 2012; Zhu 2015).

3 Production of Biofuels From Microalgae

3.1 Biodiesel

In recent decades, lipid production for biodiesel synthesis has been a recurring issue in microalgal biotechnology. In most applications, the lipids are triacylglycerides, which are found in various species of microalgae (Vanthoor-Koopmans et al. 2013). Biodiesel can be obtained from various raw materials using a process called transesterification (or esterification) with supercritical fluids or by catalysis with acid, alkali or enzymes (Fukuda et al. 2001).

Biodiesel is obtained through the conversion of lipids in the methyl or ethyl esters of fatty acids using methyl or ethyl alcohol, respectively (Xuan et al. 2009). After transesterification, the final reaction mass is composed of two phases that are separated by decanting or centrifugation. The heavier phase is crude glycerine, which contains excess alcohol, water, and impurities that are inherent in the raw material. Due to the low solubility of the glycerol esters, this type of separation typically occurs quickly. The phase containing water and alcohol is subjected to evaporation, which eliminates these volatile constituents from crude glycerin through the liquefaction of these vapors in a suitable condenser. The esters are centrifuged and dehumidified, which results in biodiesel that meets the established technical standards (Ma and Hanna 1999).

The results in Unpaprom et al. (2015) indicate that the *Scenedesmus acuminatus* strain can be a valuable candidate for biodiesel production, because it led to lipid productivity (84.4 mg L^{-1} d^{-1}). With pH adjustment (between 6.8 and 7.2) and artificial wastewater, *Chlorella zofingiensis* presented a biomass productivity of 66.9 mg L^{-1} d^{-1}, a lipid productivity of 37.5 mg L^{-1} d^{-1} and a biodiesel yield of approximately 20% (Zhu et al. 2014). The *Spirulina* genus is little studied for use in biodiesel production due its lipid concentration (with a maximum of approximately 12.0% w w^{-1}, as found by Rosa et al. 2016). However, *Spirulina* cultivations can be used in large facilities due to their hardiness to changes in pH, temperature and light, as verified by Morais et al. (2009). Biodiesel obtained from *Spirulina platensis* (8.6% w w^{-1} of lipids) showed approximately 50% saturated fatty acids, 41% unsaturated fatty acids and 74% yield. The lower percentage of unsaturated fatty acids in microalgae biodiesel makes it more stable. Thus, in comparing *Spirulina* biodiesel with palm and tallow biodiesels (both of which have 43% saturated fatty acids and 57% unsaturated fatty acids) *Spirulina* produces a better biodiesel (Nautiyal et al. 2014). Systems that were designed for commercial biodiesel production using microalgae have reported productivities of 12,000 L ha^{-1} $year^{-1}$ (Seambiotic Israel) and 98,500 L ha^{-1} $year^{-1}$ (HR BioPetroleum Inc. Hawaii). These values are more attractive when considering that the algal ponds or bioreactors for microalgae cultivation are situated on non-arable land (Schenk et al. 2008).

3.2 Bioethanol

Microalgal biomass is a promising raw material for bioethanol production, because it contains carbohydrates such as cellulose and starch. Both polysaccharides can be converted into fermentable sugars for the production of biofuel. The production of bioethanol from microalgae begins with cultivation, followed by cell harvesting. Later, carbohydrates are released into the liquid medium through cell rupture (Mussatto et al. 2010; Chen et al. 2013). This step can be performed by physical methods that include high-pressure homogenization, microwave, ultrasound, and heat (Halim et al. 2012; Hernández et al. 2014, 2015) or by the dissolution of cell walls using enzymes. This step is essential because most of these compounds are trapped inside the cell wall (such as cellulose and hemicellulose) or inside the cell in the form of starch (Domozych et al. 2012). Soon after, the carbohydrates are separated extracting with water or an organic solvent. Once extracted, these compounds can be used for bioethanol production using technology similar to that used for other raw materials involving the saccharification and fermentation processes (Matsumoto et al. 2003).

Before fermentation, saccharification must be completed to hydrolyze the carbohydrates, making them fermentable. Saccharification may be chemical (acid or alkali) or enzymatic (Chen et al. 2013). The fermentation of microalgal biomass can occur with separate or simultaneous saccharification and fermentation (Balat et al. 2008). Fermentation occurs when yeasts metabolize carbohydrates under anaerobic conditions, turning them into bioethanol and CO_2. During the final step of the process, bioethanol is distilled and purified to remove water and other compounds that may be present in the final product (Mussatto et al. 2010).

The cultivation conditions (i.e., pH, temperature, illuminance, and primarily the concentration of the cultivation medium) influenced the microalgae composition directly. In this context, the removal of phosphorus and the reduction of the nitrogen concentration (0.125 g L^{-1}) in a *Chlorella minutissima* culture promoted the highest carbohydrate yields by microalga (Margarites and Costa 2014). The yield in ethanol was greater when using pretreated biomass from *Chlamydomonas reinhardtii* (51.2 $g_{ethanol}$ $g_{glucose}{}^{-1}$) compared to the reference medium (20.1 $g_{ethanol}$ $g_{glucose}{}^{-1}$) (Nguyen et al. 2009). Harun et al. (2010a) showed that *Chlorococcum* sp. has the potential to be used as a substrate for bioethanol production in the context of biorefineries. This research showed a significantly higher ethanol concentration (3.83 g L^{-1}) when the fermentation occurred with microalgae biomass that contained no lipids.

3.3 Biohydrogen

Biohydrogen is produced during anaerobic phases and under sulfur deprivation. Its advantage is its direct photoautotrophic production through the biophotolysis of

microalgae biomass (Kruse and Hankamer 2010), such that intensive energy is not expended in downstream processing. The remaining biomass from biophotolysis can be used for biogas production to improve yield, saving costs (Sialve et al. 2009). When there is a hydrogen production phase, half of the algal biomass is generated during the previous phase. Biohydrogen recovery is conducted in a separate reactor through membrane separation. Fluctuations in hydrogen production can be controlled in stable storage facilities for the discharge of hydrogen for its intended use (Meyer and Weiss 2014). The use of biomass, biodiesel, biomethane, and biohydrogen for energy production has aroused great interest (Wijffels and Barbosa 2010). Biohydrogen production by microalgae can be economically competitive with other technologies with this same purpose, such as wind-powered electrolysis (Ni et al. 2006).

The hydrolysis of biomass with lactic acid bacteria followed by the indirect photolysis of microalgal biomass led to photosynthetic H_2 production, resulting in H_2 yields of up to 8 mol H_2 mol^{-1} of starch–glucose from *Chlamydomonas reinhardtii* (66% starch conversion efficiency) (Ike et al. 1997). H_2 production occurs under the dark fermentation of *Chlorella vulgaris* and *Dunaliella tertiolecta* microalgae at 60°C (Carver et al. 2011). The integration of dark fermentation (with *Clostridium butyricum*) with the mixotrophic cultivation of *Chlorella vulgaris* presented maximum biohydrogen production (205 mL L^{-1} h^{-1}) and no discharge of chemical oxygen demand because the byproducts of dark fermentation and the cultivated biomass were reused (Liu et al. 2013). A *C. vulgaris* biomass concentration of 10 g L^{-1} with enzymatic pretreatment at thermophilic conditions increased the H_2 yields sevenfold (135 mL_{H2} $g_{volatile\ solids}{}^{-1}$) and the energy yield from the H_2 fermentation (1.46 kJ $g_{volatile\ solids}{}^{-1}$), which is higher than the reported values in the literature (Wieczorek et al. 2014).

3.4 Biomethane

Biomethane can be generated by the anaerobic digestion of microalgae biomass in combination with other materials. Raw biogas contains approximately 55–75% w w^{-1} biomethane, 20–35% w w^{-1} CO_2 and 3,000–5,000 ppm hydrogen sulfite (H_2S). Depending on the feedstock, biomethane can be present in the biogas, with trace gases such as nitrogen (N_2), ammonia (NH_3), sulfur dioxide (SO_2), and hydrogen (H_2) (Kao et al. 2012). For several species, its high proportion in proteins decreases the carbon/nitrogen ratio to an average of 10.2 for freshwater microalgae (Elser et al. 2000). More biomethane is obtained from microalgae after the using the same approach to produce hydrogen because the levels of starch and lipids in the residue are higher (Doebbe et al. 2010).

He et al. (2016) applied biopretreatment with *Bacillus licheniformis* bacterial culture in *Chlorella* sp. cultivations, which increased the chemical oxygen demands (27%), volatile fatty acids (27%), and biomethane production by 13.5%. Low concentrations of *Clostridium thermocellum* can increase the bacterial cell

disruption of *C. vulgaris* and increase biomethane production by approximately 5% (Lü et al. 2013). Another way to increase the biomethane production would be through the thermal pretreatment of algal biomass to increase its digestibility (Yen and Brune 2007). However, during this process, the energy consumed during pretreatment can be higher than the energy obtained through the combustion of the resulting biomethane.

4 Other Bioproducts of Microalgal Origin

4.1 Polysaccharides

In addition to the production of bioethanol, polysaccharides of microalgal origin are high-value compounds. These carbohydrates have applicability in food and textile products as stabilizers, emulsifiers, lubricants, and thickeners (Arad and Levy-Ontman 2010). The sulfated polysaccharides in the walls of microalgae cells exhibit pharmacological activity and may be used in the development of medicines and cosmetics. These polysaccharides have activity as antioxidants (Chen et al. 2010), anti-inflammatories (Matsui et al. 2003), antivirals (Kim et al. 2012), and anticancer compounds (Fedorov et al. 2013). Studies on animal feed have shown that rodents whose diets are supplemented with low concentrations of red-microalgae polysaccharide have significantly reduced serum levels of cholesterol, triglycerides and low-density lipoprotein (Dvir et al. 2009), with no evidence of toxic side effects (Arad and Levy-Ontman 2010).

The cell wall of *Porphyridium* sp. contains sulfated polysaccharides that have been shown to have antiviral and anti-tumor activities. This anti-tumor activity has been demonstrated against myeloid Graffi tumors, both in vitro and in vivo, in hamsters. Antiviral activity was observed against herpes simplex virus (type 1 and 2) and varicella zoster virus (Huleihel et al. 2001). *Porphyridium* sp. polysaccharides are not toxic compared to other sulfated sugars, making them promising candidates for use in the development of pharmaceuticals and cosmetics, especially for topical applications (Arad and Levy-Ontman 2010).

4.2 Polyunsaturated Fatty Acids

Polyunsaturated fatty acids (PUFAs) are essential nutrients that cannot be synthesized by higher eukaryotes and are thus high-value compounds in the food market. Among all the PUFAs that are produced commercially by microalgae, eicosapentaenoic acid (EPA, 20: 5, ω-3) and docosahexaenoic acid (DHA, 22: 6, ω-3) are of particular interest due to their bioactivity. Studies have shown that microalgae may contain high concentrations of EPA and DHA, and these organisms are considered

potential sources of these fatty acids (Spolaore et al. 2006). When microalgae are subjected to stress conditions such as nutrient depletion, pH changes, and high salinity, the fatty acids can be found as triacylglycerols (Hu et al. 2008). In addition, microalgae contain two essential fatty acids, EPA (C20:5) and DHA (C22:6) as well as other ω-3 fatty acids that most other cultures do not have. These essential fatty acids must be ingested because they are not synthesized through animal metabolism. Therefore, it is very important to search for sources of EPA and DHA (Vanthoor-Koopmans et al. 2013).

4.3 Proteins, Protein Hydrolysates, and Peptides

The high protein content of microalgae has caused these organisms to be considered an unconventional protein source. The concentration of this macromolecule is based on crude protein, which is commonly used in the assessment of food and feed (Becker 2004). For example, the cyanobacterium *Spirulina* has a high protein content (greater than 65% w w^{-1}) as noted by Rosa et al. (2015).

Protein hydrolysates are monomers obtained during protein processing. They include peptides, which may be composed of two or more amino acids joined by peptide bonds. Hydrolysis may occur by chemical (acidic or basic), physical or enzymatic means. When it occurs enzymatically, hydrolysis is used to improve the physical, chemical, and functional food properties and the absorption of the matrix, without damaging the nutritional value (Lisboa et al. 2014). Lisboa et al. (2014) achieved a higher degree of protein hydrolysis in *Spirulina* sp. LEB 18 (55.3%) and *Chlorella pyrenoidosa* (52.9%) using the Protemax 580L enzyme.

Microalgae have been recommended as an alternative protein source due to their high protein contents and amino acid profile (Romero García et al. 2012). *Spirulina*, *Chlorella,* and *Dunaliella salina* are used in diets as sources of bioactive peptides due to their high protein content and good nutritional value (Castro-Puyana et al. 2013). Some studies have reported that peptides from various food sources have antihypertensive, antioxidant, anticancer, antimicrobial, immunomodulating, and cholesterol-reducing effects (Shahidi and Zhong 2008). Derivatives of marine bioactive peptides have attracted attention because of their numerous health benefits, such as their use in functional foods, nutraceuticals, and pharmaceuticals with therapeutic potential in the treatment or prevention of various diseases (Kim and Kang 2011).

4.4 Pigments

Pigments are specific molecules that are present in photosynthetic microorganisms to collect light energy. These compounds can be divided into chlorophylls, carotenoids, and phycobilins (Masojídek et al. 2013). Chlorophyll a is the primary

pigment in all photosynthetic organisms, followed by accessory pigments such as chlorophyll b, chlorophyll c, chlorophyll d, carotenoids, and phycobilins (Yen et al. 2013).

Chlorophyll (Chl) is made up of a tetrapyrrole ring containing a central magnesium atom and a long-chain terpenoid alcohol. All Chl have two major absorption bands, 450–475 nm (blue or blue-green) and 630–675 nm (red), which result in their characteristic green color (Masojídek et al. 2013). Chl is used primarily as a food additive to provide a green color to foods such as pasta, pesto and wormwood products. Pastes that contain *Spirulina* Chl are sold as a substitute for the strong roots used in Japanese cuisine (Hosikian et al. 2010), as an ingredient in deodorants and in pastilles to fight bad breath (Koller et al. 2015).

Carotenoids are yellow, orange, or red pigments. They are found in most photosynthetic organisms, are insoluble in water, and are usually linked to the membranes inside the cells within the chloroplast in most algae or in photosynthetic cyanobacterial lamellae (Xiao et al. 2011). Astaxanthin is used for the dietary supplementation of salmon and trout to give an orange pigment to their meat (Hussein et al. 2006). This compound can be found in microalgae biomass and orange crustaceans (Yen et al. 2013). Astaxanthin is a carotenoid with powerful antioxidant activity in comparison to β-carotene (with ten times the activity) and α-tocopherol (with five hundred times the activity) (Dufosse et al. 2005). In this context, astaxanthin has high potential for scavenging free radicals, consequently acting against cancer and several inflammatory processes (Hussein et al. 2006).

Blue phycobiliproteins are produced by the genus *Spirulina* and red-microalgae, such as the genera *Porphyridium*, *Rhodella,* and *Bangia*, at a kilograms-per-year scale. The global market was estimated at approximately $50 million in 1997, with prices ranging from US $3 to $25 mg^{-1} (Milledge 2011).

Spirulina is a unique mixture of carotenoids, zeaxanthin, and phycocyanin (Chopra and Bishnoi 2007), which create this organism's blue-green color. Green algae such as species in the *Chlorella* genus have the highest chlorophyll content of all the microalgae. *Chlorella* is marketed worldwide, with an annual output of 2,000 tons of dry powder, representing 50% of the *Chlorella* produced in Taiwan (Milledge 2011). *Spirulina* is rich in C-phycocyanin, which is generally present in cyanobacteria, rhodophytes, and cryptophytes (Glazer 1994). As a result, phycobiliproteins can improve the efficiency of photosynthetic microalgae. Among the various phycobiliproteins, C-phycocyanin is the predominant pigment present in microalgae, whereas allophycocyanins are a minority (Moraes et al. 2010). Therefore, C-phycocyanin has been investigated as a natural food coloring, replacing the synthetic pigments (Mary Leema et al. 2010) and acting as a colorant in cosmetic products such as lipstick and eyeliner (Sarada et al. 1999), among other uses. Furthermore, C-phycocyanin may be used in the pharmaceutical and cosmetic industries due to its antioxidant, anti-inflammatory, and neuroprotective properties (Romay et al. 2003).

4.5 Biopolymers and Polyhydroxyalkanoates

Biopolymers are considered plastics of biological origin, and they are analogous to polymers of petrochemical origin. The primary advantage of bioplastics is their rapid and total degradability when deposited in the environment (Somleva et al. 2013). The polyhydroxyalkanoates (PHAs) are a group of materials that are involved in carbon storage, energy, and stress metabolites; they accumulate in prokaryotic microorganisms, typically in response to unfavorable growth conditions. Poly-β-hydroxybutyrate (PHB) is the simplest constituent of these biopolymers; it is a natural thermoplastic polyester with properties similar to those of plastics derived from petroleum. The synthesis of PHA and PHB was demonstrated in cyanobacteria such as *Spirulina* sp. (Morais et al. 2015b), *Nostoc* sp. (Sharma and Mallick 2005), and *Synechocystis* sp. (Panda et al. 2006).

A study by Martins et al. (2014) showed that *Spirulina* sp. LEB 18 produced a maximum of 44% w w^{-1} of biopolymer when grown autotrophically in Zarrouk medium modified through the reduction of 90% w w^{-1} $NaNO_3$ and 50% w w^{-1} $NaHCO_3$. For the same microalgal strain grown with Zarrouk medium diluted to 20% v v^{-1} with water from the Mangueira lagoon, Morais et al. (2015a) found a maximum PHB concentration of approximately 30% w w^{-1}. The PHA concentration produced by *Spirulina subsalsa* increased by approximately 12% w w^{-1} when the NaCl concentration was doubled (Shrivastav et al. 2010). In mixotrophic cultures of *Synechocystis* sp. PCC 6803, the synergistic effect of phosphorus deficiency and limitations in the gas exchange increased the PHB concentration up to 38% w w^{-1} (Panda and Mallick 2007).

The cyanobacterium *Nostoc muscorum* showed 8% w w^{-1} PHB when grown photoautotrophically, but when cultivated mixotrophically with the addition of 0.4% w v^{-1} glucose and acetate, the polymer concentration increased to 35% w w^{-1} (Sharma and Mallick 2005). Bhati and Mallick (2012) also reported the accumulation of other copolymers such as poly (3-hydroxybutyrate-co-3-hydroxyvalerate) and P(3HB-co-3HV) in *Nostoc muscorum* up to 58 to 60% w w^{-1} under phosphorus and nitrogen deficiency and supplementation with 0.4% w w^{-1} acetate and valerate. P(3HB-co-3HV) is a copolymer that gives plastics a lower hardness compared to poly-3-hydroxybutyrate (Borowitzka 2013).

5 Residual Biomass of Microalgae

Biofuels produced from a wide range of raw materials have the potential to reduce GHG emissions (Bucy et al. 2012). In particular, the use of microalgae as a feedstock has been highlighted in recent years (Zhu and Ketola 2012). Studies have been conducted on the commercialization of microalgae-based biodiesel production (Singh et al. 2011), but this production is still expensive (Yusuf et al. 2011). For the relevant processes to be economically feasible, an alternative is the use of most of

the cellular components (proteins, carbohydrates, and lipids) in biorefineries (Demirbas 2009).

The residual biomass that forms after lipid extraction for biodiesel production is high in protein and carbohydrates. These proteins may be used for animal feed (Mata et al. 2010), and carbohydrates can be exploited as a raw material for bioethanol production (Harun et al. 2010a). Rashid et al. (2013) presented a brief overview of residual biomass applications using microalgae. According to these authors, this biomass can be converted into biofuel and other products, including a food supplement for cattle, biosorbents of heavy metals from industrial effluents and dyes.

To utilize the cellular components fully, the residual material that remains after the production of biodiesel and ethanol can be subjected to pyrolysis to convert it into bio-oil (Mirsiaghi and Reardon 2015). Thangalazhy-Gopakumar et al. (2012) performed a catalytic pyrolysis of green algae to produce hydrocarbons using the HZSM-5 catalyst in a fixed-bed reactor. These authors identified negative attributes of the bio-oil from algae, such as high levels of nitrogen and oxygen, which were reduced by using HZSM-5 as a catalyst.

Kim et al. (2015) investigated the pyrolysis characteristics and kinetics of *Dunaliella tertiolecta* by examining the residual biomass obtained after the extraction of lipids and saccharification. The initial biomass of *D. tertiolecta* was composed of 22.0% w w^{-1} lipids, 40.5% w w^{-1} carbohydrates, 27.2% w w^{-1} proteins and 10.3% w w^{-1} ash. After lipid extraction, the residual biomass was analyzed. The carbohydrate, protein, and ash contents then increased by 51.9, 35.0, and 13.1%, respectively. Saccharification was performed on the residual biomass, yielding a second residual biomass composed primarily of protein (67.7%, w w^{-1}). Finally, the authors concluded that the residual biomass remaining after the pyrolysis of *D. tertiolecta* could contribute to a microalgal biorefinery.

Both the biomass of microalgae in nature and the residual biomass from lipid extraction may be used for ethanol production (Harun et al. 2010a). To facilitate fermentation, several methods have been used to convert biomass to fermentable substrates, including thermal and alkaline conditions (Yang et al. 2011), acid treatment (Talukder et al. 2012) and sonication (Jeon et al. 2013). Mirsiaghi and Reardon (2015) evaluated the effects of different cell wall carbohydrate hydrolysis methods on the residual biomass of *Nannochloropsis salina*. The authors found that hydrolysis with sulfuric acid increased the yield of the released carbohydrates, while the highest rate of carbohydrate release was obtained through hydrochloric acid treatment. Thus, the authors concluded that the hydrolysates that were generated from the residual biomass could be used as a substrate for producing biofuel and bioproducts by fermentation.

The carbohydrate content and composition of the residual biomass obtained from lipid extraction depends on the microalgal species (Schwede et al. 2013), cultivation phase (Fernández-Reiriz et al. 1989), growth conditions (Sukenik et al. 1993; Schwede et al. 2013) and lipid extraction process (Yang et al. 2011). According to Park et al. (2012), residual biomass defatted with organic solvent (hexane) is recommended for biogas but not for bioethanol production. After the

lipids and pigments are extracted from the microalgal biomass, they can be used as substrates for fermentation to produce biohydrogen. Yang et al. (2011) observed a decrease in biohydrogen production when the initial concentration of residual microalgal biomass was increased from 4.5 to 45 $g_{volatile\ suspended\ solids}\ L^{-1}$.

Nobre et al. (2013) used the biomass of the Nannochloropsis sp. microalga as raw material to produce fatty acids for biodiesel, biohydrogen and high value-added compounds. The microalgal biomass, which contained high levels of fat and pigments (primarily carotenoids), was subjected to supercritical extraction with CO_2. It was possible to extract 45% w w^{-1} of the lipids and recover 70% w w^{-1} of the pigments. Furthermore, the authors found that the remaining biomass was effectively usable as a raw material for the production of biohydrogen through fermentation by *Enterobacter aerogenes*, resulting in a biohydrogen yield of 60.6 g mL^{-1} of dry biomass.

Extraction residues of microalgal biomass have potential for use as feedstock in biorefineries. The use of residual biomass contributes to the production of renewable energy and the sustainable development of a microalgae biodiesel industry. The focus has been lipid extraction; nevertheless, for the more efficient use of the biorefinery concept, the residual biomass from the extraction of other macromolecules, such as proteins, carbohydrates, and several others biocompounds, can be further explored.

6 Final Considerations

Microalgae have the potential to produce biofuels and high value-added bioproducts with various applications. Using microalgal biomass within a biorefinery concept contributes to the production of clean energy and products for nutrition and health, bringing benefits to society such as food safety, employment and income. In addition, the cultivation of microalgae can positively influence the environment, for example, through sustainable land use and reductions of GHG emissions and wastewater. Therefore, as shown in this review, the development of a microalgae-based biorefinery is a promising, environmentally friendly, and self-sustaining alternative.

Acknowledgements The authors thank the following Brazilian development agencies: the CAPES (Coordination for the Improvement of Higher Education Personnel), R&D Program ANEEL-Eletrobrás CGTEE (National Agency of Electric Energy-Company of Thermal Generation of Electric Power), CNPq (National Council of Technological and Scientific Development), and MCTI (Ministry of Science, Technology and Innovation), and the Program to Support the Production of Publication Academic/PROPESP/FURG/2015.

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Microalgae Mixotrophic Growth: Opportunity for Stream Depuration and Carbon Recovery

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Abstract Mixotrophic cultivation is a trophic way in which microalgae can drive photoautotrophy and heterotrophy and can utilize both inorganic and organic carbon sources. In mixotrophic cultures the two metabolic processes, photosynthesis for photoautotrophy and aerobic respiration for heterotrophy, affect each other, contributing to synergistic effects and improving the growth rate, with an enhancement of biomass productivity. This chapter explores the main characteristics and different possible applications of microalgae culture under mixotrophy. The growing of microalgae under mixotrophy represents a promising application to remove efficiently the organic compounds, nitrogen and phosphorus present in high concentrations in urban, livestock and agro-industrial wastewaters. Therefore, such a treatment is considered an effective method of waste remediation. Additionally, mixotrophic cultivation provides a cost-effective microalgae biomass for use as alternative energy such as biofuel. Another possible application of mixotrophy is the production of high added value molecules (such as LC-PUFA, EPA and DHA, astaxanthin) by using specific growth media and different agro-industrial by-products as the C source (i.e. glycerol and glucose). In this chapter, biomass production and the target products obtained by using different C source and microalgae strains described in the literature will be discussed. Integrating algal biomass production with wastewater treatment and with the utilization of by-products has the potential to become a highly selective strategy for enhancing the cost effectiveness and environmental sustainability of algal cultivation.

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B.N. Tripathi and D. Kumar (eds.), *Prospects and Challenges in Algal Biotechnology*, DOI 10.1007/978-981-10-1950-0_5

1 Introduction

Mixotrophic cultivation is a trophic way that allows microalgae to live via photoautotrophy and heterotrophy using both inorganic and organic carbon sources (Kang et al. 2004). The assimilation of organic carbon is influenced by light and occurs through photosynthesis, while the assimilation of organic compounds takes place through aerobic respiration, which depends on the availability of organic carbon (Hu et al. 2012a).

Mixotrophy in ecosystem is a rule rather than an exception: it is wide spread among prokaryotes and protists (Matantseva 2013). The main hypothesis is that the capability for mixotrophic growth might be the backup alternative of obtaining energy when photosynthesis is impossible, for instance when illumination is insufficient, or other limiting factors occur in oligotrophic environment, providing significant competitive advantages to the organisms. Mixotrophic nutrition in protists is a prominent example of cellular mechanisms providing interaction of unicellular organisms with their environment and has a great ecological importance (Jones 1994; Sanders 1997; Esteban et al. 2010).

Some scientists consider the specific growth rate of microalgae mixotrophically cultivated as the sum of the specific growth rates obtained by cultivating microalgae under photoautotrophic and heterotrophic modes (Marquez et al. 1993). Others suggested that some kind of synergetic mechanisms are involved, and this data are consistent with the highlighted mechanism overcoming photoinhibition in mixotrophy (see section Mixotrophy and light). They consider that in mixotrophic cultivation photosynthesis and aerobic respiration act in synergy, enhancing biomass productivity (Yu et al. 2009; Acién et al. 2013). In mixotrophic cultivation of microalgae the use of organic compounds allows algae do not entirely depend on photosynthesis: light is not an outright restricting variable for the growth of microalgae. Microalgae cultivated under mixotrophic mode showed an improve in their growth rate, a reduction in growth cycle and biomass losses in dark hours and an augmentation in biomass productivity due to the supplementation of photoautotrophy with carbon substrates. (Park et al. 2012). Some strains, under mixotrophic condition, are able to enhance lipid content as percentage on dry weight obtaining a lipid productivity useful for microalgal biodiesel production. Finally, the CO_2 emitted by microalgae trough aerobic respiration can be caught and reused by microalgae mixotrophically cultivated. This mechanism enhances inorganic carbon availability for microalgae and thus further improves biomass and lipid productivities (Mata et al. 2010).

2 What Triggers Mixotrophy

By now is not clear why, how and at which moment do autotrophs begin assimilating the dissolved organic substance. Simple approach would suggest that autotrophy is used when light and mineral nutrients (N and P) are no limiting

factors, whereas light and nutrient limiting conditions combined with the presence of available organic substrates should promote heterotrophic nutrition (Hansen et al. 1997; Li et al. 2000). However, data have shown that regulation of mixotrophy is based on less linear mechanisms. Thus, it was found out that good illumination in protists can induce not only photosynthesis, but also phagocytosis, while the presence of organic substrates is able to accelerate the inorganic carbon fixation, thus supplying the organism with necessary biogenic elements (Moorthi et al. 2009; Burkholder et al. 2008). Many studies suggest that other factors such as temperature, CO_2 saturation, oxygen concentration, life cycle stage, selection and growing media composition can play the trigger role. Currently regulation principles of mixotrophy in microorganisms have not been fully understood, and it can be merely stated that most likely there do not exist universal laws for all mixotrophic organisms (Matantseva and Skarlato 2013). Although it is not clearly understood how these factors affect mixotrophy metabolism, a comprehensive consideration and effective control of these variables may lead to obtain ideal cultivating conditions maximizing productivities (Wang et al. 2014). The main identified factors affecting mixotrophic responses are the carbon source and the illumination regime. Mixotrophy is triggered, first of all, by the presence of an organic carbon substrate in adequate amount and quality (Hu et al. 2012b). Illumination regime also plays an important role in mixotrophic cultivation. Even if less susceptible toward light than photoautotrophic culture, illumination is a very critical factor impacting productivities of mixotrophically developed microalgae. Wavelengths of 600–700 nm (red light) are most suitable for photosynthesis, whereas wavelengths of 400–500 nm (blue light) may enhance the comprehensive growth rate of microalgae in mixotrophy (Wang et al. 2007; Carvalho et al. 2011). Some authors also report that low light intensity encourages mixotrophic cultivation (Legrand et al. 1998; Graneli et al. 1999; Stoecker et al. 2006; Burkholder et al. 2008). Moreover, the CO_2 supply seems to affect mixotrophic triggers, in fact carbon dioxide is the main limiting factor for algal growth and its overabundance strongly improves photosynthetic productivity (Sforza et al. 2010); thus, CO_2 supply is required to reach a high productivity even in mixotrophic conditions. However, it seems that the microalgae are not able to consume organic carbon with an excess of CO_2 concentration in the medium (Sforza et al. 2012), thus to pull mixotrophic growth a sharp control of CO_2 supply is required.

3 Mixotrophy and Light

Microalgal growth is linked to light intensity and the generalized Light Response Curve (LRC) relating algal growth (P) to light intensity (I) (P-I) has the trend shown in Fig. 1. LRC can be divided into three phases: (I) photolimitation phase, wherein growth rate raises linearly with increasing light intensity, (II) photosaturation phase wherein growth rate is moderately independent from light intensity,

and (III) photoinhibition phase wherein growth rate decreases with increasing light intensity (Ogbonna et al. 2000). Since most algal species become light saturated at a low fraction of peak solar-light intensity, much potentially useful solar energy is essentially wasted for photosynthesis.

For example, the light saturation constants for microalgae *Phaeodactylum tricornutum* and *Porphyridium cruentum* are 185 and 200 μE m^{-2} s^{-1}, respectively (Mann and Myers 1968; Molina Grima et al. 2000). Vejrazka et al. (2011, 2012) reported as photosaturation range a light intensity ranged from 100 to 500 μE m^{-2} s^{-1}. The usual midday outside light intensity in equatorial areas is about 2000 μE m^{-2} s^{-1}, and value around 1000–1500 μE m^{-2} s^{-1} can be reached in sunny days at higher latitude location.

Over a definite value of light intensity, beyond light saturation, a further increase in light level, not only does not increase photosynthesis, but also reduces the biomass growth rate (Fig. 1). This phenomenon is called photoinhibition. Microalgae are photoinhibited at light intensities little higher than the light level at which the specific growth rate peaks. Removing photoinhibition or its deferment to higher light intensities can significantly increase the average daily growth rate of algal biomass. Because of light saturation, and subsequent photoinhibition the biomass growth rate and thus total yield, is much lower than theoretically possible.

3.1 Sensitivity to Photoinhibition

Microalgae cultivated in mixotrophic conditions are less sensitive to photoinhibition than those cultivated in photoautotrophic mode, independently by illumination intensity. Photoinhibition of *Spirulina* sp. was observed for light intensities over

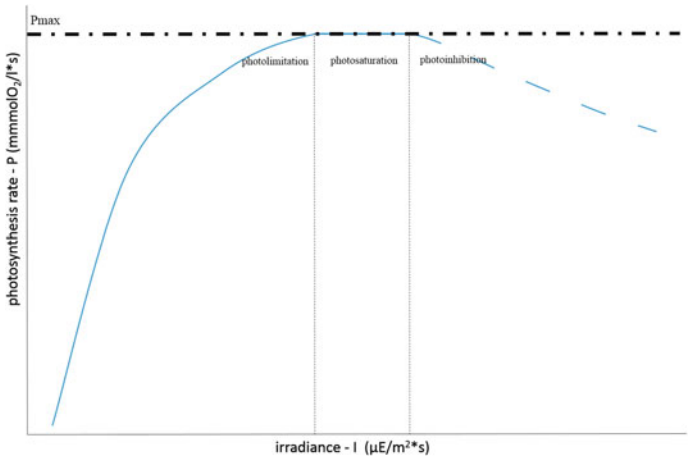

Fig. 1 Light response curve for microalgae

50 W m^{-2} in photoautotrophic system, while under mixotrophic cultivation inhibition was not detected up to light intensities of 65 W m^{-2} (Chojnacka and Noworyta 2004). Moreover, after photoinhibition occurred, microalgae under mixotrophic cultivation recovered more quickly and to a higher extent.

It is generally assumed that photoinhibition results from two factors:

1. the incapability of the photosynthetic apparatus to employ the exceeding light energy absorbed by the photosynthetic antenna: there is a disparity between the quick rate of photon catch by the light-harvesting apparatus and the slower downstream rate of photosynthetic electron transfer (Perrine et al. 2012);
2. The production of reactive oxygen species (ROS): in order to oxidize water, microalgae harvest light energy, exchanging electrons closeness to molecules such as singlet oxygen or triplet chlorophyll a, thus producing harmful reactive oxygen species (ROS). When ROS amasses and caused more damage than that can be adjusted, algae are subjected to oxidative stress.

3.2 Protection Mechanism Against Photoinhibition

The reduced sensibility of mixotrophic cells to photoinhibition has been attributed to five main mechanisms:

1. Higher Cell concentration: the mixotrophic culture allows obtaining higher biomass production and thus higher cell density, since radiant energy is not the only promoting factor for the growth, but the carbon provides an additional energy input. The higher cell density of microalgae grown in mixotrophy determines a greater shadowing and thus a lower average exposure of each cell to light radiation, i.e. the same radiant energy can be distributed to a higher number of cells in mixotrophic condition, thus limiting possible damage. Microalgae growth under mixotrophic conditions have a 20–40% higher growth rate in comparison with photoautotrophic cultures at any light intensity provided (Vonshak et al. 2000).
2. Re-balance of light-dependent and enzymatic-dependent reaction. The light-catch reaction is quicker than the subsequent enzyme-mediated reactions, thus the maximum rate of photosynthesis must be checked by the concentration of one of the Calvin cycle's enzymes (Sukenik et al. 1987). A deficiency of electron sinks downstream of photosystem I (e.g. carbon fixation) can result in accumulation of electrons in the electron transport flow and consequently an increased risk of ROS production and photoinhibition (Niyogi 2000). An increase in Calvin cycle activity, due to the abundance of organic carbon, can lead to increase in the consumption of reduction power (Vonshak et al. 2000).
3. Rapid repair of damage to photosystem II: damages from photoinhibition are promptly repaired, depending on the environmental conditions and the

physiological conditions of the cell, through the action of D1 protein. PSII is susceptible to be damaged by high irradiation. Ohad et al. (1984) suggested that the turnover of D1 protein is part of a repair system to replace the damaged function centers with newly synthesized protein D1 thus restore the normal PS II activity. The reestablishment from the photoinhibition is not just a counter reaction to stressing condition, but it is an active anabolic process aimed to synthesize great amount of D1 protein. The more rapidly recovery rate founded in mixotrophic cultures was ascribed to higher metabolic activity (Vonshak et al. 2000), and this mechanism had been already highlighted by Cheung et al. (1998) that obtained greater recovery from photoinhibition in mixotrophy. Anyway the increased recovery was cancelled when chloramphenicol (a protein inhibitor) was applied to culture: in this case mixotrophic and autotrophic recovery was the same.

4. Reduction in the dimension of the light-harvesting antenna and reduction in chlorophyll content (Beckmann et al. 2009; Liu et al. 2009). This mechanism reduces the light adsorbing capacity of individual cell, increasing light penetration in deep layers of photobioreactors and reducing heat dissipation of absorbed light energy, thereby increasing photosynthetic efficiency in high light and high cell density culture (Eriksen 2008). This mechanism is highly effective considering not the individual efficiency, but the whole production system (high densities PBR). The less effective performance of individual cell allows to protect the single cell and to distribute light thus achieving a better performance of the whole system.
5. Oxygen decrease in the culture medium: high dissolved oxygen concentration in close photobioreactors might accelerate oxidative reactions.

   – Increased oxygen consumption. The oxygen produced by photosynthesis is released in the culture medium. In algae culture exposed to high Photon Flux Densities (PFD) the dissolved oxygen concentration in the medium can reach above 200% of air saturation, limiting condition for algae growth and chlorophyll synthesis (Ugwu et al. 2007). Some microalgae strains are not able to survive in significantly O_2^- oversaturated environment longer than 2–3 h (Pulz 2001). Cells growing mixotrophically, thanks to the respiration reactions promoted by carbon abundance, consume oxygen and allow a considerable decrease of the concentration of dissolved oxygen in the culture medium and the entire photobioreactor, thus reducing photoxidative damage (Chojnacka and Marquez-Rocha 2004).
   – Decreased oxygen production: Roach et al. (2013) showed that *C. reinhardtii* in mixotrophic conditions produced less O_2 by thylakoids than those from photoautotrophic cultures due to destabilization in secondary quinone acceptor promoting direct non-radiative charge recombination events that do not drive to oxygen generation.

3.3 Light Limiting Condition

Taking into consideration light limiting condition, i.e. the left-most part of the Light Response Curve, mixotrophically cultivated microalgae are less susceptible to different illumination intensities, and they can better adapt to and recover from daytime light variations, which would mitigate the cost of artificial illumination. Mixotrophic cultivation showed a low light sensitivity, that is particularly useful for growing microalgae at high cell densities or with shady media like wastewaters, in those events where light frequently becomes a limiting factor (Li et al. 2012b).

4 Productivity and Energy Balance

4.1 Biomass Productivity

Since the use of organic substrates, microalgal biomass productivity under mixotrophic conditions is generally much higher than that both in photoautotrophy and heterotrophy (Wan et al. 2011; Xiong et al. 2010; Ogbonna et al. 2000). The biomass productivities of *Nannochloropsis oculata, Dunaliella salina, Chlorella sorokiniana, Spirulina platensis*, and *Scenedesmus obliquus* cultivated in mixotrophy with supplementation of glucose were 1.4, 2.2, 2.4–4.2, 3.8, and up to 8.7 times higher than the same microalgae phototrophically cultivated (Chen et al. 1997; Mandal et al. 2009; Wan et al. 2011). The biomass productivity of *Phaeodactylum tricornutum* cultivated under mixotrophic mode adding glucose, acetate, and glycerol increase of 1.5-, 1.7-, and 2.5-fold, respectively, compared to that obtained with phototrophic cultivations (Liu et al. 2009). Under well-controlled mixotrophic conditions, some microalgae strains can reach synergistic effect achieving biomass productivities higher than that obtained with heterotrophic cultivation (Yu et al. 2009). Bhatnagar et al. (2011), examined the biomass productivities of *Chlamydomonas globosa, Chlorella minutissima*, and *Scenedesmus bijuga* under autotrophy, mixotrophy and heterotrophy conditions. Results indicated that biomass productivities of *Chlamydomonas globosa, Chlorella minutissima*, and *Scenedesmus bijuga* mixotrophically cultivated adding 1% (w/v) of glucose, were 9.4, 6.7, and 5.8 times higher than those cultivated in autotrophy condition and were 3.0, 2.0, and 4.4 times of those cultivated in heterotrophy. Compared to autotrophy and heterotrophy, the high microalgal biomass productivity under mixotrophic mode contributes to a higher biomass production, allowing to reaching a better economic viability for large-scale plants of microalgae production.

4.2 Lipid Productivity

Lipid productivity is determined by both biomass productivity and lipid content, which can be expressed as follows:

$$\text{Lipid productivity} = \text{biomass productivity} \times \text{lipid content}$$

Combined effects of biomass productivity and lipid content should be evaluated to achieving the highest possible lipid productivity. Mixotrophic cultivation of microalgae seems to be very interesting because allow to achieve higher biomass productivity with limited lipid content reduction. Indeed, the lipid productivity of mixotrophic cultivation of *Nannochloropsis* sp. with glycerol was improved by 40–100% compared to autotrophic control (Probir et al. 2011). Mixotrophic cultivation of *Nannochloropsis oculata, Dunaliella salina* and *Chlorella sorokiniana,* with glucose supply, allowed to obtain lipid productivities that were by 1.1–1.6, by 1.8–2.4 times and by 4.1–8.0 times of those under photoautotrophic cultivation, respectively (Chojnacka and Marquez-Rocha 2004). According to Mandal and Mallick (2009), *Scenedesmus obliquus* cultivated mixotrophically supplying by 1.5% (weight/volume) of glucose could achieve a lipid productivity as high as 270 mg l^{-1} day^{-1}, almost 50 times of that reached with photoautotrophy cultivations. Moreover, by comparing the growth of *Chlorella protothecoides* in mixotrophy with glucose supplementation, with growth of those cultivated in heterotrophy, it was observed a 69% higher lipid productivity with mixotrophic mode (Xiong et al. 2010). Liang et al. (2009) studied the lipid production of *Chlorella vulgaris* under the three cultivation conditions. Results showed that the lipid productivity of *Chlorella vulgaris* mixotrophically cultivated with the supplementation of glucose at 1% (w/v), was 1.5 times and 13.5 times higher than that obtained by heterotrophy and autotrophy modes respectively.

4.3 Energy Efficiency

The performance of microalgae culture can be compared through the efficiency of conversion (E) by which all the energy supplied to the culture is utilized for biomass production. To do that the inlet energy supplied to the system (radiant energy and chemical energy) is compared with the energy content in the microalgae biomass (chemical energy) i.e.

$$\text{Energy in biomass}/(\text{energy from light} + \text{energy from organic carbon})$$

Yang et al. (2000) reported biomass yields on the supplied energy (Y_{SE}) equal to 0.00924 g kJ^{-1} for mixotrophy, 4 times higher than that recorded for autotrophy finally finding that Y_{SE} was the lowest in the autotrophic cultivation; opposite the mixotrophy gave the most effective utilization of energy for biomass production.

Table 1 Energy conversion efficiency

Cultivation mode	Ec (%)	Et (%)	References
Autotrophy	0	1.2	Ren et al. (2014)
Mixotrophy	45.7	14.6	
Heterotrophy	34.1	34.1	
Autotrophy	0	3.5	Yang et al. (2000)
Mixotrophy	40.5	18.5	
Autotrophy	0	3–8	Molina Grima et al. (1997)

Ec: conversion efficiency of chemical energy
Et: conversion efficiency of the total supplied energy (light and organic carbon)

The efficiency of conversion from light energy to biomass in autotrophy was around 3.5% (Table 1), opposite in mixotrophy the total efficiency of conversion into biomass was equal to 18%. This data are confirmed by Ren et al. (2014) that founded a total efficiency of conversion of energy in autotrophy equal to 1.2% and 14.6 for mixotrophy (Table 1). Not surprisingly, the efficiency of conversion in microalgae cultured under autotrophy was the lowest due to the inefficient conversion of light energy into biomass. Average data recorded for light conversion efficiency into chemical energy range from 1 to 8% at lower photon flux densities (Molina Grima et al. 1997). If we consider only the amount of energy provided as organic carbon to algae the conversion efficiency is quite variable, from 18% (Yang et al. 2000) to 45% (Ren et al. 2014) to be compared with 58% recorded for unicellular microorganism, e.g. *Candida utilis* (Trinh et al. 2009). It is interesting to underline (Table 1) that conversion efficiency of organic carbon in algae is boosted with light in mixotrophy; in fact the total carbon conversion efficiency is 34.4 in heterotrophy but is 45.7 for mixotrophy, being that consistent with synergistic effects previous reported for mixotrophy.

Considering all the energy balance, i.e. energy from light and from organic carbon, mixotrophically grown microalgae show the highest energy conversion efficiency.

Due to rapid light attenuation by the suspending cells, shadowing and light distribution heterogeneity occurs inside the photobioreactor: i.e. the limitation of light energy is the most frequently problem observed in practical cultures of photosynthetic cells.

For maximum energy use efficiency, the light intensity should be homogeneously distributed in the entire photobioreactor, keeping the light intensity between the critical and the saturation ranges. In a practical photobioreactor, simultaneous existence of complete dark, light limitation, light saturation and light inhibition zones inside the same photobioreactor is a common phenomenon. Light energy supply and its efficient utilization is the greatest scientific and technological challenge in research and development on cultivation of photosynthetic microorganisms.

In mixotrophic cultures, the energy source form organic carbon is homogeneously distributed inside the bioreactor so it is possible to exploit the heterotrophic metabolism occurring in some photosynthetic cells that are in light limiting

condition. This carbon supply can counterbalance the very heterogeneous light distribution in photobioreactors and rebalance the energy flux within the microalgae cells thus gaining a better energy efficiency.

4.4 Advantages of Mixotrophic Cultivation

In addition to the ecological significance mixotrophy is an interesting productive opportunity due to the possibility: (I) to depurate organic downstream, and (II) to increase the production of valuable compounds using organic carbon so overcoming light limitation or eventually softening light inhibition, in any case increasing production.

Microalgae mixotrophically cultivated allow obtaining higher productivities with the same organic carbon amount with respect to heterotrophy that depends only on organic carbon sources.

5 Mixotrophy Exploiting Wastewaters

Great volumes of wastewaters from industries processing agricultural raw materials, livestock, industries and wastewaters from domestic treatment plants are annually dumped to aquatic ecosystems worldwide (Dareioti et al. 2009; Bhatnagar and Sillanpää 2010). These effluents are characterized mainly by a high concentration of organic matter, nitrogen and phosphorous, and a variable pH (Drogui et al. 2008). Both the flow rate and characteristics of these wastewaters are industry specific and can vary throughout the year (Dareioti et al. 2009). Uncontrolled disposal of such effluents into natural water bodies often results in surface and groundwater contamination and other environmental problems such as eutrophication and ecosystem imbalance (Drogui et al. 2008; Posadas et al. 2014). Therefore, it becomes necessary to develop low cost and environmentally friendly methods for the treatment of wastewaters. The initial purpose of introducing microalgae to wastewater treatment process was to accomplish tertiary treatment with particular attention to nutrients removal; in addition, it was detected that microalgae could effectively depurate sewage from organic pollutants (Wang et al. 2010a) and increase productivity of biomass thanks to organic carbon, thus improving productivity and depuration.

While the ability of algae to remove N and P from wastewater has been extensively studied (Woertz et al. 2009; Liang et al. 2013; Gentili 2014), how algae growth relates to the organic carbon content in wastewater medium had less research attention (He et al. 2013; Tian-Yuan et al. 2013). The combination of microalgae cultivation with wastewater as growth medium is a valid joining for waste remediation and, also, to reduce total costs of microalgal biomass production (i.e. for biofuel, see Sect 5.5).

Different wastewater streams, including digested and/or undigested animal slurry, concentrated and un-concentrated municipal wastewater, and agricultural raw material, have been used to support mixotrophic microalgae growth for biomass production coupling with a wastewater treatment. Microalgae from the Chlorella and Scenedesmus genus present a very good adaptation in wastewaters and can reach high biomass productivity; in fact, these two microalgae genus are the most commonly used strains for wastewater treatment (Li et al. 2012a; Craggs et al. 2013). Li et al. (2011) also found that algae strain Chlorella sp. cultivated in centrate wastewater stream, produces comparable biomass and lipid productivity with those obtained growing on standard medium showing excellent adaptation on wastewaters and great potential to synthesize valuable compounds (Li et al. 2012a).

5.1 Urban Wastewater

Human beings generate every year billions tons of domestic wastewater (FAO Aqua-stat), containing average carbon nitrogen and phosphorus amount as indicated in Table 2. Municipal wastewater can be generally divided in: (I) primary wastewaters (PW), i.e. wastewaters after primary settling; (II) secondary wastewaters (SW), which is wastewater after secondary treatment by activated sludge; (III) tertiary wastewaters (TW) after tertiary treatment (N and P uptake) has been performed; (IV) centrate wastewater (CW), generated after dewatering, sludge by centrifuge.

Primary treatment of wastewater aims at removing particulate in the sewage through grids or sedimentation. Secondary treatment decreases the biochemical oxygen demand (BOD) in wastewaters by oxidizing the organic material and ammonium. This process involves both heterotrophic bacteria and protozoa and it is often performed in aerated cisterns with so-called activated sludge. The bacteria degrade the organic compounds and the protozoa graze the bacteria, and in both cases they produced carbon dioxide and water starting from the organic matter. Tertiary wastewater treatment mainly aims at removing nitrogen and phosphorus.

Algal systems have usually been used to take out pollutants in wastewaters (Lavoie and De la Noüe 1985; Martin et al. 1985; Oswald 1988). The ability of microalgae to uptake organic carbon, justify and support the attempt to use microalgae also for secondary treatment of wastewaters and the treatment of

Table 2 Chemical composition of urban wastewaters (range)

Type of wastewaters	COD ($mg\ L^{-1}$)	N ($mg\ L^{-1}$)	P ($mg\ L^{-1}$)	References
Primary wastewater	150–500	33–100	4–25	Zhou(2014); Henze (2008); Samorì et al. (2014)
Secondary wastewater	24–34	8–15	0.5–50	Zhou (2014); Bunani et al. (2015)
Centrate	2250	131	200	Zhou (2014)

centrate. Organic carbon in sewage is mainly present in form of carbohydrates, lipids, proteins, amino acids and volatile acids thus they are readily available carbon sources, suitable for microalgae uptake. The inorganic compounds are primarily sodium, calcium, potassium, magnesium, chlorine, sulphur, phosphate, bicarbonate, ammonium salts and heavy metals (Lim et al. 2010).

Centrate is high in carbon content (around 1000 mg L^{-1}) and proved to be favourable to selected mixotrophic genus such as *Chlorella* sp., *Heynigia* sp., *Hindakia* sp., *Micractinium* (Zhou et al. 2011). In the case of primary wastewater and centrate the mechanisms for nutrients removal in wastewater by microalgae include photosynthetic absorption and/or chemosynthetic assimilation by mixotrophic/heterotrophic metabolic pathway (Table 3).

Auxenochlorella protothecoides UMN280 derived from municipal wastewater plant showed high nutrient removal efficiency and also high growth rate and lipid productivity. Batch cultivation showed maximal removal rates for total nitrogen, total phosphorus and chemical oxygen demand (COD) over 59%, 81% and 88% respectively, with a good growth rate (0.490 d^{-1}), high biomass productivity 269 mg L^{-1} d^{-1}, and high lipid productivity (78 mg L^{-1} d^{-1}) (Zhou et al. 2012). The presence of organic carbon may counter balance for the shortage of CO_2 dissolved in the growing medium, which is often the limiting factor for the growth of microalgae in low cost growing systems (e.g. open ponds). The presence of organic carbon replaces the presence of CO_2, both by heterotrophic metabolism (organic carbon assimilated as such) that by autotrophic (carbon employed in the form of CO_2 produced after mineralization by microorganisms present in the culture medium or produced by enhanced respiration of microalgae heterotrophic

Table 3 Phycodepuration of urban wastewaters

Microalgae strain	Wastewaters	Removal			References
		COD (%)	N (%)	P (%)	
Auxenochlorella protothecoides UMN280	Concentrated municipal wastewater	88	59	81	Zhou et al. (2012)
Euglena sp.	Sewage treatment plant	–	93	66	Mahapatra et al. (2013)
Chlorella vulgaris	Synthetic sanitary sewage	78.7	74.6	72.8	Xu et al. (2013)
Botryococcus braunii	Domestic wastewater	–	79.6	100	Sydney et al. (2011)
Auxenochlorella protothecoides	Concentrated municipal wastewater	81.4	73.6	75.1	Hu et al. (2012b)
Chlorella sp.	Centrate wastewater	70	61	61	Min et al. (2011)
Chlorella sp. and *Scenedesmus* sp.	Domastic wastewater	90	100	–	Hammouda et al. (1995)

metabolism). The increased algal growth due to organic carbon finally allows for greater purification of the wastewater. Ledda et al. (2015a, b, c) proved that *N. gaditana* could be cultivated using centrate as the only nutrients source at percentages under 30%, whereas higher percentage resulted in ammonia inhibition. Nitrogen depuration reduced from 85 to 63% with the increase of centrate concentration in the culture medium and the decrease in biomass productivity. Phosphorus remediation from the culture medium was 85% whatever the centrate percentage in the culture medium demonstrating a phosphorus limitation into the cultures. The use of centrate was confirmed as a valuable method for reducing microalgae production costs and for enhancing process performance.

5.2 *Livestock Wastewaters*

In the past few decades global agriculture and livestock activities have increased rapidly in conjunction with the growing food demand of the global population (FAO 2014). Technological innovations have led to profound structural changes and improvements in the agro-zootechnical sector, increasing the productivity efficiency, but at the same time raising negative environmental implications associated with the expansion of this sector. Agriculture and livestock sectors produce large amounts of effluents particularly animal manure wastewaters that are extensively available all over the world and can origin severe pollution issue if not properly managed (Zhou 2014). Every year, in the United States, around 35 million dry Mg of livestock wastes are generated, while in the EU-27 more than 1,500 million fresh Mg of livestock wastes are produced every year (Choi et al. 2014). Nowadays, the management of livestock wastes mainly includes the production of bioenergy from livestock wastes through biological (i.e. anaerobic digestion) or thermo-chemical processes, composting for agricultural employment and combustion for heat and electricity production (Zhu and Hiltunen 2016). The hurdle is the developing sustainable approaches to manage, recycle and add value to agriculture wastewaters reducing any negative impacts on the environment. In this context, microalgae-based processes represent a cost-effective technology for the well-management of livestock wastewaters (de Godos et al. 2009; Mulbry et al. 2008). Effluents from poultry, piggery and dairy farms contain huge quantities of nitrogen, phosphorus and organic carbon (Table 4) in both particulate and soluble forms; the composition is strictly related to farming practices and animal nutrition (Bernet and Bèline 2009). Another interesting stream able to support microalgae growth is the digestate produced by the anaerobic digestion process (see Sect 5.2.1). Some authors sustain the possibility to recovery and reuse nutrients from digestate through the cultivation of microalgae. (Franchino et al. 2013; Ledda et al. 2015a, b, c). The high nutrients concentration, high turbidity and possible contamination by microorganism are the main challenges to overcome for the microalgae agro-wastewaters treatment feasibility. Many works indicated ammonia content higher than 100 mg L^{-1} as toxic concentration (Collos and Harrison 2014)

Table 4 Chemical composition of livestock wastewaters

Effluents	PH	TS	TN	TP	COD	References
		(mg L^{-1})	(mg L^{-1})	(mg L^{-1})	(mg L^{-1})	
Liquid pig slurry	6.3	26000	2880	710	3189	Misselbrook et al. (2002)
Anaerobic Digestate	7.97	80000	2940	50	9906	Ledda et al. (2013), (2015a, b, c)
Pig manure	8.37	211100	6295	3194	54498	Li et al. (2012a)
Chicken manure	6.95	550000	24035	10120	49045	Ho et al. (2013)
Dairy manure	7.5	117000	1884	551	13161	Liu et al. (2011)

even if several microalgae species showed a good tolerability. For example, *Chlorella sorokiniana* exhibited a growth inhibition at an ammonia concentration above 210 mg L^{-1} (Munoz et al. 2005) whereas *Spirulina platensis* was inhibited at 150 mg L^{-1} (Ogbonna et al. 2000). Sepúlveda et al. (2015) reported the absence of inhibition for *Nannochloropsis gaditana* cultures at an ammonia concentration of up to 334 mg L^{-1}. As livestock wastewater contains amount of ammonia (Table 4) at least one order of magnitude higher, the commonly adopted strategy is to dilute the stream to reach the proper nutrients level requirement for algae growth (Zhou et al. 2012) contemporary reducing the shading effect due to the dark colour of the effluents.

Trials shown in Table 5 demonstrate that different microalgae species are able to grow on livestock wastewaters determining high nutrient removal efficiency. *Chlorella* is the most renowned genus used for nutrient removal in wastewaters, thanks to the excellent adaptation of these microalgal species on this substrate (Li et al. 2012a). Franchino et al. (2013), reports that *Chlorella vulgaris* presented the highest removal capacity of ammonium in a diluted 1:10 digestate sample (derived from a mix of cattle manure and raw cheese way), with a 96% removal efficiency, and it was also observed that only the 4% of ammonia was stripped. This author sustains that *C. vulgaris* has higher growth rate than the other two strains used *Scenedesmus obliquus* and *Neochloris oleoabundans,* with a μ (day^{-1}) of 0.64, 0.49 and 0.27 $days^{-1}$, respectively. Similar results were observed by Wang et al (2010b) that assessed that *Chlorella vulgaris* grown in anaerobic digestate dairy manure removed ammonia, total nitrogen, total phosphorus, and COD by 100%, 75.7–82.5%, 62.5–74.7%, and 27.4–38.4%, respectively. As regards to carbon removal, Kim et al. (2000), showed that *Spirulina platensis* grown on different concentration of swine waste for 12 days in batch culture, was able to reduce 80–90% of COD, the highest detected. De Godos et al. (2009) reports that a microalgal-bacteria consortium have reached a total Kjeldahl nitrogen and COD removals of 88 ± 6% and 76 ± 11% in a high-rate algal ponds (HRAPs) cultured on diluted swine manure for 245 days with an hydraulic residence time of 10 days.

In considering the use of wastewater to support microalgae growth, another key issue is the negative effect of bacterial contamination on microalgal biomass survival and quality leading to an important limitation in the scale-up of cultivation of

Table 5 Phytodepuration of livestock wastewaters

Microalgae strain	Wastewaters	Removal			References
		COD (%)	N (%)	P (%)	
Neochloris oleoabundans	Agro-zootechnical digestate	–	99.9[a]	96.9[c]	Franchino et al. (2013)
Chlorella vulgaris		–	99.9[a]	96[c]	
Scenedesmus obliquus		–	83.7–92.4[a]	96.1[c]	
Chlorella sp.	Digested manure	27.9–38.4	100[a]; 75.7–82.5[b]	62.5–74.7[d]	Wang et al. (2010b)
Chlorella pyrenoidosa	Primary piggery wastewater	36.5–55.4	91.2–95.1[a] 54.7–74.6[b]	31–77.7[d]	Li et al. (2012a)
Chlorella sp. *UMN271*	Fermented liquid swine manure	62.45–72.58	26.73–99.9[a] 12.99–55.85[b]	79.08–88.56[c]	Hu et al. (2012a, b)
Chlorella sorokiniana and aerobic bacteria	Liquid fraction of pig manure	62.3	82.7[a]	58[c]	Hernandez et al. (2013)
Ourococcus multisporus	Piggery wastewater	–	19[b]	–	Abou-Shanab et al. (2013)
Nitzschia cf. pusilla		–	17[b]	–	
Chlamydomonas mexicana		–	62[b]	28[d]	
Scenedesmus obliquus		–	60[b]	–	
Chlorella vulgaris		–	51[b]	–	
Spirulina platensis	Swine waste	80–90	67–93	70–93[c]	Kim et al. (2000)
Microalgal-bacterial consortium	Diluted swine manure	76	88	10 [c]	de Godos et al. (2009)
Scenedesmus obliquus	Piggery wastewater	42	36[a]	27–65[d]	de Godos et al. (2010)
Chlorella sorokiniana		42–47	21–25[a]	20–54[d]	
Euglena viridis		51–55	34–39[a]	28–60[d]	
Neochloris oleoabundans	Anaerobic effluents from pig waste	–	98[a]	98[c]	Olguin et al. (2015)
Chlorella sp.	Digested swine manure	61–63	95–98	85–99%	Ledda et al. (2015a, b, c)

[a] Ammonium (NH_4^+)
[b] Total nitrogen
[c] Phosphates (PO_4^{3-})
[d] Total phosphorous

microalgae using wastewaters. This issue could be overcome by using and/or combining different strategies, e.g. isolating wild microalgae strains that tolerate substrates, such as livestock slurries. Ledda et al. (2015a, b, c) isolated a wild

microalgae strain from digested pig slurry to evaluate differences in growth and remediation performances in sequential digestate liquid fractions sampled from a full-scale digestate-treatment plant. The isolated *Chlorella* proved to be a strong strain, capable of reducing about 95–98% of N-NH_4^+ and 61–73% of COD, while micronutrients were almost completely removed.

5.2.1 Anaerobic Digestion Plant and Microalgae: A Perfect Model for Exploiting Downstream

Anaerobic digestion (AD) is used to stabilize organic waste streams (mainly livestock slurries, but also by-products and waste) producing biogas (50–75% CH_4 and 25–50% CO_2) that can be used to produce renewable energy in place of fossil fuel-derived energy. The downstream of biogas production are: digestate rich in N P and residual COD, heat, CO_2. Recently new paradigm for AD has been developed in order to overcome problem related to the AD cost versus subside (Manenti and Adani 2014) and develop an exemplary model of circular economy. In this paradigm the biogas plants has been indicated as the facility unit to build a diffused bio-refinery model (Manenti et al. 2016), producing different goods: bio-methane, and nutrient (N and P), organic nutrients (COD), CO_2 and heat useful to produce 3rd generation biomass (microalgae). This approach allows diversifying biogas products, reducing biogas cost and increasing circular economy implementation. Ledda et al. (2015a, b, c) considered the feasibility of integrating microalgal biomass production with anaerobic digestion process, in particular a dairy cattle manure and subsequent digestate treatment, was used to sustain algae growth. In this way, it was possible to reduce the cost of slurry treatment and, meantime, improve the energy balance of the whole process. Real biogas plant and his digestate-treatment units were constantly checked for energy, mass and nutrient balances. Microalgae production of *Scenedesmus* sp. provided the use of untreated ultra-filtered digestate as substrate of the biomass growth. Results showed a growth inhibition of the selected strain with a percentage of digestate over 10%, below this value productivity was up to 124 mg L^{-1} d^{-1}. Biomass composition was influenced by the composition of the culture medium, with proteins content being positive correlated with ammonia concentration. Finally, it was demonstrated that was possible integrate microalgae production with anaerobic digestion process, producing 166–190 Mg y^{-1} of valuable microalgal biomass (Fig. 2).

5.3 *Agro-Industrial Wastewaters*

The compositions of agro-industrial wastewaters are industry specific and can vary significantly during the year considering the seasonal variation of the processed materials (Table 6).

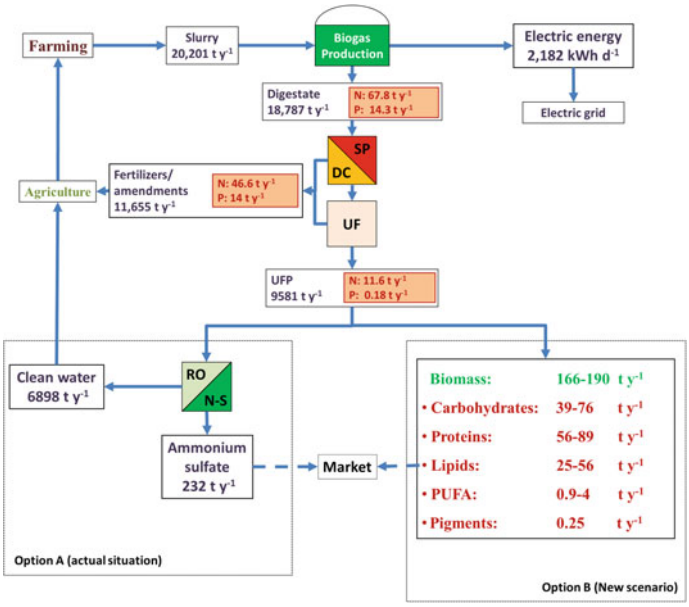

Fig. 2 Anaerobic digestion plant as model for exploiting downstream. Biomass production from microalgae in option B box (Ledda et al. 2015a, b, c); SP (screw press separator), DC (decanter centrifuge), UF (ultrafiltration), RO (reverse osmosis), N-S (ammonia stripping). With kind permission of Elsevier

Despite their relevance, little attention has been given to the treatment of agro-industrial wastewaters (Posadas et al. 2014). Nutrient reduction in agro-industrial wastewaters varies greatly depending on their composition; in the literature are reported reductions of COD, N and P ranging from 30–40% to nearly 100% (Table 7). Dumas et al. (1998) studied the ability of cyanobacterium *Phormidium bohneri,* to remove dissolved inorganic nutrients from fish farm effluents. Average efficiencies of ammonia nitrogen and orthophosphate removal was 82% and 85% respectively. Blier et al. (1995) examined the cyanobacterium *Phormidium bohneri* and of the endogenous microalga *Micractinium pusillum* in term of growth and nutrient removal efficiency for the bio-treatment of a cheese factory anaerobic effluent. Ammonia was almost totally removed in four days when *Phormidium bohneri* and *Micractinium pusillum* are used, although the kinetics of removal were different for both species. Removal of phosphorus after four days of culture was only 33% for *Micractinium,* and 69% with *P. bohneri*. Phang et al. (2000) grew *Spirulina* on anaerobically digested palm starch factory wastewaters: the percentage of reductions in COD, ammonia and phosphate reached 98.0%, 99.9% and 99.4% respectively. More recently (Hongyang et al. 2011) *Chlorella pyrenoidosa* was cultured in soybean processing wastewater. The alga was able to remove about 78% of soluble chemical oxygen demand (COD), 89% of ammonium

Table 6 Chemical composition of agro-industrial wastewaters

Type of wastewater	COD (mg O_2 l^{-1})	N (mg l^{-1})	P (mg l^{-1})	References
Cheese factory anaerobic effluent	1500	125	80	Blier et al. (1995)
Fish farm wastewater	152	–	–	Dumas et al. (1998)
Soybean processing	13215	267.1	56.3	Hongyang et al. (2011)
Potato processing	1536	33.7	4.2	Hernandez et al. (2013)
Potato processing	872	69	6	Posadas et al. (2014)
Fish processing	1016	82	6	
Animal feed production	2557	197	27	
Coffee manufacturing	22752	766	59	
Yeast production	3163	703	7	
Digested palm starch processing	1340	40	21	Phang et al. (2000)
Dairy industry wastewaters	6000	18.45	5.58	Kothari et al. (2013)

Table 7 Phycodepuration of agro-industrial wastewaters

Microalgae strain	Wastewaters	Removal			References
		COD (%)	N (%)	P (%)	
Phormidium bohneri	Fish farm wastewater	66	82	85	Dumas et al. (1998)
Chlorella pyrenoidosa	Soybean processing	78	89	70	Hongyang et al. (2011)
Chlorella sorokiana + aerobic sludge	Potato processing	85	>95	81	Hernandez et al. (2013)
Phormidium (71%), Oocystis (20%) and Microspora (9%)	Potato processing	54	60	–	Posadas et al. (2014)
	Fish processing	64	74	–	
	Animal feed production	49	80	–	
	Yeast production	33	50	–	
	Coffee manufacturing	56	80	–	
Phormidium bohneri	Cheese factory anaerobic effluent	–	98	69	Blier et al. (1995)
Micractinum pusillum		–	97	33	
Spirulina platensis	Palm starch processing	98	99.9	99.4	Phang et al. (2000)
Chlamydomonas polypyrenoideum	Dairy industry wastewaters	64	90	70	Kothari et al. (2013)
Chlorella vulgaris	Textile wastewater	38–62	44	33	Lim et al. (2010)

nitrogen and 70% of total phosphate. Hernandez et al. (2013), treated potato processing wastewaters with a microalgae-bacteria consortium of *Chlorella pyrenoidosa* and aerobic sludge. The removal efficiency was very high, indeed ammonium was almost exhausted (decrease >95%), phosphorous removal efficiency was 80.7%, while total COD was utilized for 85% if its initial content.

Kothari et al. (2013) tested the remediation ability of *Chlamydomonas polypyrenoideum* on dairy industry wastewater Results obtained indicate that *C. polypyrenoideum* can growth well on dairy industry wastewater in comparison with BG-11 growth medium. Algae grown on dairy industry wastewater were able to use carbon for biomass generation (64% of uptake) decreased the pollution load of nitrogen (90%) and phosphate (70%) in 10 days of treatment.

Posadas et al. (2014) tested the potential of algal–bacterial symbiosis for the removal of carbon, nitrogen and phosphorus from five agro-industrial wastewaters: potato processing, fish processing, animal feed production, coffee manufacturing and yeast production. The highest removals of nitrogen (85%) and total organic carbon (64%) were observed for fish processing wastewaters while the maximum $P\text{-}PO_4$ removal achieved was 89% in undiluted potato processing wastewaters. The authors moreover observed that the biodegradable TOC was, in most cases, the limiting component in the treatment of the wastewaters evaluated.

Dumas et al. (1998) observed a maximum growth rate of *Phormidium bohneri* cultivated on fish farm effluents of 0.06 mg d.m. day^{-1}. These values were expected because the concentration of inorganic nutrient were very low respect to Blier et al. (1995) that examined the cyanobacterium *Phormidium bohneri* and of the endogenous microalga *Micractinium pusillum* in term of growth and nutrient removal efficiency for the bio-treatment of a cheese factory anaerobic effluent. *Phormidium bohneri* showed higher growth rate ($\mu_{max} = 0.62\ d^{-1}$) and biomass yield (329 mg dm l^{-1}) than that of *M. pusillum* (0.35 d^{-1} and 137 mg dm l^{-1}) over 4 days. Phang et al. (2000) cultivated *S. platensis* using wastewater from the production of palm starch as mixotrophic medium. The specific growth rate was 0.51 1/d and the biomass productivity was 14.4 g m^2 d^{-1}. The highest protein, carbohydrate and lipid content of the biomass were 68%, 23% and 11% respectively. Hongyang et al. (2011) reported an average biomass productivity of 0.64 g L^{-1} d^{-1} with a lipid productivity of 0.40 g L^{-1} d^{-1} using fed-batch culture. Hernandez et al. (2013) treated potato processing wastewaters using microalgae-bacteria consortium, biomass production achieved 18.8 mg DW l^{-1} d^{-1}, and the microalgae lipid content was 30.2%. Kothari et al. (2013) used *Chlamydomonas polypyrenoideum* to perform a phyco-remediation process on dairy industry wastewater for biodiesel production. After 10 and 15 days, they compared the microalgal biomass obtained in mixotrophy with that obtained in autotrophy (BG-11 as control), and both times algae growth on dairy wastewater presented the highest lipid content.

5.4 Full-Scale Reactors for Phytoremediation

Algal high-rate ponds (HRPs) were developed starting from the 1950s as an alternative to oxidation ponds for BOD, suspended solids, and pathogen removal. HRPs are raceway shape ponds 30,100 cm deep, equipped with a pump to mix wastewater. Hydraulic retention time (HRT) is very short (4–10 days) depending on climatic conditions (Rawat et al. 2011). HRP can be used as a combined secondary/tertiary system for wastewater treatment. Microalgae in these ponds can produce high protein biomass at a rate of 10–20 g m^{-1} d^{-1}, productivities an order of magnitude greater than land crops (Oswald 1995). Productivities of up to 50 t ha^{-1} y^{-1} are achievable in these systems, consuming approximately 0.57 kWh kg^{-1} BOD removed. In contrast, a much higher amount of energy in the range of 0.80–6.41 kW h kg^{-1} BOD removed was consumed by mechanical aerated ponds. HRPs are actually used to treat urban wastewater and waste from pig farms and digestate (Olguín et al. 2003; Fallowfield et al. 1999) and for the treatment of the effluent from aquaculture system (Pagand et al. 2002). Approximately, half of the total energy consumed by wastewater treatment plants is used for supply oxygen to the bacteria consortium to oxidize the organic carbon into CO_2 and nitrogen into N_2, which are then released to the atmosphere. Alternatively, microalgae can produce O_2 by taking up the CO_2 released by the bacteria thus reducing both the energy consumption and the CO_2 released to the atmosphere (Acièn et al. 2013). The technology based on microalgae-bacteria consortiums needs large surface areas and favourable environmental conditions, thus for this technology is very hard to replace current processes based on activated sludge (Gómez-Serrano 2015) (Table 8).

Table 8 Comparison of energy input for wastewater depuration

Treatment	Energy consumption (kWh m^{-3})	References
Standard secondary + tertiary treatment	0.2–1.6	Rawat et al. (2011)
	1.05	Singh et al. (2012)
Microalgae secondary + tertiary treatment (HRP)	0.14	Rawat et al. (2011)
Standard tertiary treatment	0.23–0.96	Acién Fernández et al. (2013)
	0.5–1.0	Gómez-Serrano et al. 2015
Microalgae tertiary treatment	0.05–0.11	Acién Fernández et al. (2013)
	0.1–0.2	Gómez-Serrano et al. (2015)

5.5 Possible Products Recovery from Wastewater Depuration: Biodiesel

The use of organic wastewaters to produce microalgal biomass present some disadvantages such as high organic and inorganic pollutants (e.g. urban and industrial wastewaters) and biological contaminants like bacteria or fungi (Pittman et al. 2011; Abinandan and Shanthakumar) which may reduce the quality and quantity of microalgae biomass (Chen et al. 1997). For this reasons the biomass produced by wastewaters depuration should be better addressed to the production of no- food products, such as biofuel. Indeed the depleting resource of petroleum fuels and the environmental concerns associated to them have generated serious necessities for alternative fuels. Microalgae family includes species that can accumulate large amounts of lipids in the form of triglycerides (TAGs) that can be turned into biofuel (Collet et al. 2014). Biodiesel from microalgae is a well known option due to its high energy density, better environmental performance compared to diesel and suitable for use in diesel vehicles with little alterations to their motors (Tan et al. 2015). However, the main problem related to the real feasibility of this application process at industrial-scale is related to the high production costs (Chisti 2007), in particular the cost related to the fertilizer and water input. For example, microalgae cultivation request a N-fertilization in the range of 0.29–0.37 kg/kg oil, which is nearly ten times higher than that for oil palm (0.048 kg kg^{-1} oil) (Lam and Lee 2012) and two fold higher that of other land plant producing oil. The integration wastewater treatment with microalgal biomass production is a promising approach to make algal biofuel production more cost effective (Clarens et al. 2010; Olguin 2012; Li et al. 2014). Yang et al. (2011) reports that the use of wastewaters could reduce the need for additional nitrogen and phosphorous sources by approximately 55%. Therefore, the possibility to use wastewaters derived from municipal, agricultural, and industrial activities like source of nutrients for microalgae cultivation could significantly reduce the operational costs of algal production systems (Lardon et al. 2009) performing an environmental service (depuration) at the same time. As described before (see Sect. 1.2), lipid's productivity is be determined by the product between the biomass productivity and the lipid content. The lipid productivity of a mixotrophically cultivated microalgae could increase up to 8 times more than photoautotrophic cultivation (Probir et al. 2011; Chojnacka and Marquez-Rocha 2004) (see data reported in Sect. 1.2). Recent studies involving the use of Life Cycle Analysis have indicated the necessity of decreasing the energy and fertilizer consumption in biodiesel process (Lardon et al. 2009; Yang et al. 2011). In conclusion the use of microalgae for bioenergy purposes (e.g. biodiesel), it's technically feasible, but still needs more considerable R&D efforts to achieve the high productivities required at low cost, could so competing with fossil diesel (Table 9).

Table 9 Microalgae grown on by-products

Microalgae strain	By-products	C source ($g\ L^{-1}$)	Biomass ($g\ L^{-1}$)	Lipid (% TS)	Carotenoid (% TS)	References
Chlorella vulgaris	Glucose	30	10	13	–	Heredia-Arroyo et al. (2011)
Chlorella vulgaris	Cheese whey	0	1.22	40	–	Abreu et al. (2012)
		10	1.98	40	–	
		10	3.58	40	–	
	Glucose + galactose	10	2.24	40	–	
Chlorella vulgaris	Glycerol + glucose	5 + 2	2.60	–	0.4	Kong et al. (2013)
	Glycerol	1	0.62	–	0.1	
Cholorella zofingiensis	Glucose	30	9	–	0.14 (Astaxanthin)	Ip and Chen (2005)
Chlorella protothecoides	Glucose	15	8.5	–	–	Wang et al. (2013)
	Glucose	17.1	8.5	29.4	–	
	Glucose	16.5	8.6	39.9	–	
	Glucose	17.1	7.6	57.3	–	
	Glucose	15.4	7.7	38.4	–	
Scenedesmus obliquus	Cheese whey	0	1.9	–	–	Girard et al. (2013)
		40	4.9	10 (PUFA)	–	
Parietochloris incisa	Glucose	0.9	1.2	–	–	
Chlorella protothecoides	Glycerol	4	2.67	–	–	Sforza et al. (2012)
Nannochloropsis salina		4	0.43	–	–	
Parietochloris incisa	Glucose	0	0.22	–	–	Tababa et al. (2012)
		0.9	0.71	8.6	–	
		0.9	1.1	7.4	–	
Nannochloropsis salina	Glucose	5.4	0.51	4.6 (EPA)	–	Xu et al. (2004)
	Ethanol	1.4	0.45	–	–	

(continued)

Table 9 (continued)

Microalgae strain	By-products	C source ($g\ L^{-1}$)	Biomass ($g\ L^{-1}$)	Lipid (% TS)	Carotenoid (% TS)	References
Chlorella marine	Glucose	2	1.45	25.4	–	Cheirsilp and Torpee 2012
Nannochloropsis sp.		2	1.2	25.5	–	
Nannochloropsis sp.	Glucose	5	1.2	31	–	Xu et al. (2004)
Phaeodactylum tricornutum	Glycerol	0.1	7.04	2.4 (EPA)	0.45	Cerón-García et al. (2006)
	Fructose	0.02	3.5	1.59 (EPA)	0.5	
	Glucose	0.05	2.2	–	–	
	Mannose	0.01	1.05	–	–	
	Lactose	0.005	0.77	–	–	
Phaeodactylum tricornutum	Glycerol	9.2	13.8	–	–	Cerón-García et al. (2013)
	Fructose	3.6	8.2	–	–	
	Glycerol	11.9	12	5.4 (PUFA)	–	
Phaeodactylum tricornutum	Glycerol	0.1	2.4	16.8 (PUFA) 2.2 (EPA)	–	Cerón-García et al. (2000)
Spirulina sp.	Glucose	1	10.2	–	10.7 (Phycocianin)	Chen and Zhang (1997)
Spirulina sp.	Acetate	–	0.91	–	–	Lodi et al. (2015)

Data in Table 9 outline interesting production, where final biomass concentration is in the range of 8–10 $g\ L^{-1}$, which is, as outlined in Sect. 4.1 more than twice the magnitude of production which can be achieved in favoruable autotrophic condition

6 Mixotrophy Exploiting Agro-Industrial By-Products

6.1 Type of By-Products Used in Microalgae Cultivation and Biomass Production

Different organic substrates can be used by microalgae to growth under mixotrophic conditions. Glucose used as carbon substrate for mixotrophic cultivation has been widely used in the cultivation of different algae strains (*Chlorella*, *Nannochloropsis, Phaeodactylum tricornutum, Spirulina* sp.*)* (Liu et al. 2009; Yang et al. 2000; Santos et al. 2011; Wan et al. 2011; Bhatnagar et al. 2011; Herredia-Arroyo et al. 2010) providing good results, but the cost of dedicated products is high for full-scale algae production: e.g. the use of glucose for mixotrophy is estimated to range around 80% of the cost of the cultivation medium (Bhatnagar et al. 2011). Alternative carbon sources such as by-product from industrial processes (glycerol), food processing (cheese whey, ethanol, wine lees) and agro-industrial chain (acetate from digestate) might be exploited as microalgae feedstock (Lee 2004; Dragone et al. 2009; Heredia-Arroyo et al. 2010; Bhatnagar et al. 2011; Heredia-Arroyo et al. 2011; Sforza et al. 2012; Liang et al. 2009).

The use of these bio-products from agro-industrial processes, higher in quality with respect to wastewaters, allows obtaining high quality algae biomass, suitable for food and fine chemicals production.

6.2 Fine Chemicals from Mixotrophic Culture of Selected By-Products

Some microalgal species can produce valuable compounds; the production and storage of these compounds is related to the growing condition of microalgae, to the light intensities (i.e. saturating condition) and nutrient availability. In some cases, algae cultivated under stress conditions start to increase the amount of molecules used to protect their cells, such as pigments and antioxidants, or other secondary metabolites (Markou et al. 2011; Skjånes et al. 2013). Some of these secondary metabolites are fine chemicals particularly interesting for food, nutraceutical pharmaceutical or cosmetic sector (Skjånes et al. 2013) and is interesting that can be effectively produced by mixotrophic culture.

6.2.1 PUFA

Microalgae produce storage lipids (neutral lipids) and structural lipids (polar lipids). The first types (storage lipids) are mainly saturated Fatty Acids (FAs) and few unsaturated FAs that are good feedstock for biodiesel production. The second type

of lipids (structural) are mainly Long Chain Poly Unsaturated Fatty Acids (LC-PUFAs), a type of essential acids crucial in the human nutrition. According to literature omega 3 LC-PUFAs eicosapentaenoic acid (EPA; 20:5 omega 3) and docosahexaenoic acid (DHA; 22:6 3), affect blood pressure, coagulation, prevent heart diseases ad it is a structural element of eyes, brain an heart tissues.

6.2.2 Carotenoids and Astaxantin

Microalgae are considered the future large source of carotenoids (Borowitzka 1988, 1992; Del Campo et al. 2000). Microalgae use carotenoids (polar lipids) as auxiliary pigments of light-harvesting complexes, i.e. different wavelengths of light not absorbed by chlorophyll are captured by carotenoids and sent to the photosystem II) at the same time they are able to dissipate excess of light energy, thus protecting the reaction center of photosynthesis (Taylor 1996; Eskling et al. 1997; Del Campo et al. 2000).

Among the most important carotenoids, lycopene, astaxanthin and lutein are used to colour food, to enhance egg pigmentation and as food and feed additives (Borowitzka 1988, 1992; Johnson and Schroeder 1995). Carotenoids are used in nutraceutical formulation to enhance health and prevent disease: e.g. β-carotene lutein and zeaxanthin are used in "cancer-preventing formulation" (Richmond 1990; Le Marchand et al. 1993; Ziegler et al. 1996).

Astaxanthin cause the pink-red coloration of the bodies of salmonids and crustaceans (Gu et al. 1997), thus it is used as aqua-feed supplement to produce pigmentation to eggs and flesh (Cordero et al. 1996). In nutraceutical formulation astaxanthin is valuable for the strong anti-oxidative activity (Miki 1991). The relevant microalgae strains for carotenoids production are Haematococcus pluvialis for astaxanthin (Boussiba and Vonshak 1991) and Dunaliella salina, for β-carotene (Avron and Ben-Amotz 1992). Haematococcus is a low growing strain, low competitive respect to other algae genera and thus subject to risk of contamination. The astaxanthin production occurs in encysted cells that have been exposed to stressing environmental conditions: high light intensity nitrogen depletion and high salt concentration (Boussiba et al. 1991; Fábregas et al. 2003; Del Campo et al. 2004; He et al. 2007).

Other microalgal genera such as Chlorella s sp. or Scenedesmus sp., are reported to produce astaxanthin (Del Campo et al. 2004). Chlorella zofingiensis is a candidate for massive and industrial production of natural astaxanthin exploiting carbon source, as it grows easily and at high concentration mixtrophically (Del Campo et al. 2004; Feng et al. 2011; Liu et al. 2012, 2014) thus allowing the industrial scale up and economical viability.

6.3 Light and Fine Chemical Biosynthesis

Literature reports that algae may increase neutral lipids biosynthesis to counteract photo-oxidative stress that occurs when light is high and reducing equivalents are in excess for photosynthesis (Hu et al. 2008). The same does not seem to apply to LC-PUFAs. Light intensity seems to regulate the cellular content of LC-PUFAs, specifically EPA, as it is a constituent of thylakoid membrane where photosynthesis occurs (Kates and Volcani 1966; Cohen et al. 1988). When irradiance is high, algae start to decrease their photosynthetic efficiency and reduce the number of thylakoid membranes

Harwood and Jones (1989) reported lower content of LC-PUFAs in high light conditions. Consistent with this hypothesis Sukenik et al. (1989) reported that *Nannochloropsis* sp. grown under low light conditions (35 μE $m^{-2} \cdot s^{-1}$), produced 40% of galactolipids (out of total lipids) with high LC-PUFAs content, opposite *Nannochloropsis* sp., grown under high light conditions (550 μE m^{-2} s^{-1}) produced lipids with lower LC-PUFAs content.

Similar results were reported by Renaud et al. (1991); they found a significant decrease in the relative abundance of EPA when cultures of *Nannochloropsis oculata* were grown at high photon densities. DHA production processes with *Crypthecodinium cohnii* using glucose as carbon source in heterotrophy (Kyle 1996) has resulted in overall productivity of DHA on glucose equal to 19 mg L^{-1} h^{-1} (de Swaaf et al. 1999) and production up to 45 mg L^{-1} h^{-1} in cultivations with acetic acid as carbon source (Ratledge et al. 2001; de Swaaf et al. 2003) proving that DHA synthesis can occur without light. Absence of light and heterotrophic condition where applied to *Phaeodactylum tricornutum* (Sukenik et al. 1989) and *Nannochloropsis* sp. (Thompson 1996) achieving EPA content up to 39% of total fatty acids, while *Thraustochytrium* (Burja et al. 2006) and *Schizochytrium limacinum* (Zhu et al. 2007) in heterotrophy reached DHA content between 30 and 40% of the total fatty acids. DHA content can be modulated controlling environmental conditions: nutrients and carbon source, nitrogen, sodium and oxygen concentrations, temperature and pH. According to Cerón-García et al. (2013), mixotrophic cultures had elevated levels of chlorophylls, carotenoids, and LC-PUFA, relative to controls. Kitano et al. (1997) found that mixotrophic growth in acetic acid effectively promotes the productivity of EPA with a high growth rate and high EPA content of the biomass in *Navicula saprophila*. Ceron Garcìa et al. (2000) reported EPA productivity of 33.5 mg L^{-1} d^{-1} was obtained in *P. tricornutum* culture carried out in 9.2 g L^{-1} glycerol. This yield was ten fold greater than the maximum EPA productivity obtained in the control.

The yield of EPA and DHA by heterotrophic culture of many species, *Crypthecodinium, Schizochytrium*, *Ukenia* has been widely investigated and production has been performed at large scale by fermentation (Chen and Chen 2006; Wen and Chen 2003). Considering specifically DHA production in mixotrophic culturing, (Ren et al. 2009) reported that *Schizochytrium* sp. *HX-308*, increased DHA content from 35 to 60% of total fatty acids following the supply of 4 g L^{-1}

malic acid. According to up to date references LC-PUFAs content is inhibited by high photon flux density and stimulated by low irradiance, thus the mixotrophic culture mode can be the optimal condition for LC-PUFAs production: mixotrophic culture are denser and less exposed to light over saturation regimen and at the same time total growth and productivity is positively affected by organic carbon.

6.4 Other Ways to Trigger Light Induced Products

Some chemicals are able of inducing oxidative response to enhance accumulation of anti-oxidant molecules in place of light (e.g. for the accumulation of astaxanthin). Kobayashi et al. (1993) demonstrated that Fe^{2+} the superoxide anion radical (a O^{2-}) and the hydrogen peroxide H_2O_2, stimulated the accumulation of astaxanthin in *H. pluvialis*. Similarly, Ip and Chen (2005) demonstrated that is possible to boost astaxanthin production of C. zofingiensis in heterotrophy without light, using sodium hypochlorite (NaClO) as oxidant agent. (Chen et al. 2009) increased of 30% the production of astaxanthin in *C. zofingiensis* using pyruvate 100 mM. Also citrate and malic acid had the same boosting activity in *C. zofingiensis*.

Again the enhancement in metabolic activity due to mixotrophy is a driving element to increased valuable compounds production.

7 Conclusions

Mixotrophic cultivation of microalgae have proved to be effective in the depuration of wastewaters, utilizing COD, nitrogen and phosphorus, to be able to significantly enhance light use efficiency reducing the sensibility to photoinhibition, to be able to produce added value molecules such as LC-PUFA, EPA and DHA, astaxanthin, etc.

Integrating algae biomass production, CO_2 mitigation using recovered CO_2 and wastewater treatment and by-products utilization is the future key strategy of circular economy to enhance the economical and environmental sustainability of algal cultivation.

In high advanced agricultural areas the development and increase of agro-industry and livestock farming activities are arising environmental sustainability concerns, as water bodies eutrophication, air pollution by ammonia volatilization and soil degradation due to over-fertilization. Every years this sector produce huge amount of agro-industrial waste stream that normally are an additional cost for the industries. These raw materials represent a renewable carbon source, contain also N, P, K and micronutrients, that could be used as a substrates to support microalgae growth, decreasing the production costs. Dual-use microalgae cultivation coupled with the reuse of agro-food waste streams and/or wastewaters treatment is an effective option in terms of freshwater protection, nutrients recovery and GHG reduction, producing benefits in term of environmental sustainability.

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Sustainable Utilization of Marine Algae Biomass for Environmental Bioremediation

Laura Bulgariu and Dumitru Bulgariu

Abstract The rapid development of anthropogenic activities has a negative impact on the environment, due to the accumulation of harmful heavy metal ions pollutants. Biosorption on low-cost materials has been intensively studied in the last years, because they offer an efficient and cost-effective alternative to the conventional methods used for the environment decontamination. Thus, numerous utilizations of marine algae biomass have been developed for the efficient removal of heavy metal ions from aqueous environments. Unfortunately, such practical applications are not economic efficient. More advantages seem to be the utilization of marine algae biomass as feedstock for energy production. But, even if the obtaining of energy from marine algae is considered a 'clean technology', the valorization of algae waste resulted both after oil extraction and low temperature combustion is still important issue for which further solutions are sought. In this context, the utilization of such marine algae wastes as biosorbent for the removal of heavy metal ions from aqueous media; besides, that will ensure that the utilization of such materials in agreement with the principles of sustainable development will be also helpful in the environment bioremediation processes. In this chapter are comparatively presented the biosorptive performances of marine algae biomass and of wastes resulted from energy production for the removal of various heavy metals ions from aqueous media.

Keywords Marine algae · Metal ions · Biosorption · Energy feedstock Environmental remediation

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B.N. Tripathi and D. Kumar (eds.), *Prospects and Challenges in Algal Biotechnology*, DOI 10.1007/978-981-10-1950-0_6

1 Introduction

The fuels production and environment protection are two major problems, which are nowadays particularly important for the sustainable development of all societies. The necessity to replace petroleum-derived fuels with renewable biofuels has become one of the most important tools which can be used for environment protection, mainly because the petroleum-derived fuels are well known to have a limited availability and also an important contribution to global warming (Azmir et al. 2013). Although until now, the biofuels production cannot overcome all market requirements, the finding of new and renewable feedstock sources for the biofuels productions has gained increasing attention. From this perspective, the marine algae seem to be one of the potential alternatives that can be successfully used in the production of sustainable energy. The most important advantages of utilization marine algae as feedstock for energy production are

- marine algae are fast-growing plants, available in high quantities in many regions;
- their growth do not require fertile land and freshwater (Demirbas and Karslioglu 2007);
- can convert solar energy into chemical energy with higher efficiency (6–8%) than terrestrial biomass (1.8–2.2%) (Jung et al. 2013);
- marine algae have a lower risk for the competition for food and energy than other crops, because only in few countries from East Asia the marine macroalgae are used as food, fertilizer, and animal feed (Bixler and Porse 2011; Borghini et al. 2014).

Therefore, many experimental studies performed until now have shown that due to their high contents of various carbohydrates, marine algae processing requires less energy that these provides.

There are two ways in which the marine algae biomass can be used for the obtaining of energy, namely: (i) extraction of oil from marine algae biomass and then its transformation in biofuels, and (ii) combustion of algae biomass through a gasification process at relative low temperatures, the resulting gases can be then used to obtain electricity. Both procedures have numerous advantages which have determined the intensification of researches in this field (Demirbas 2009; Patel et al. 2016; Ripoll et al. 2016).

Although the obtaining of energy from marine algae is included in the category of 'clean technologies', several environmental drawbacks must still be solved. The most important is the valorization of algae waste resulted both after oil extraction and low temperature combustion, which are usually discharged as waste, or incinerated. A promising alternative for such algae waste could be their use as biosorbent for the removal of heavy metals, and such utilization will be helpful for

environmental protection. Besides finding of a use for such waste, the removal of heavy metals using such low-cost materials have important benefits for environment, and these have been recently demonstrated by Life Cycle Analysis studies (Dominguez-Ramos et al. 2014; Maul et al. 2014).

Various heavy metals ions are used in numerous industrial activities, such as: textile, chemical, pigments, storage batteries, plastics, mining, electroplating, smelting, metallurgical processes, etc. (Lyer et al. 2005; Han et al. 2006), due to their technological importance, and are included in the category of persistent environmental pollutants, because cannot be destroyed or degraded (Mehta and Gaur 2005; Park et al. 2010). In addition, due to their mobility, persistency, and accumulation tendency, their presence in natural water ecosystems has serious ecological and human health consequences, when the tolerance levels are exceeded (Aklil et al. 2004; Aydin et al. 2008). Therefore, to prevent the deterioration of environment quality, legislation governing the levels of heavy metals, discharged from industrial waste stream is becoming progressively stricter (Directive 2000/60/EC).

Under these conditions, it has become imperative to maintain or even to improve the quality of environment, the removal the heavy metals from industrial effluents, and this was imposed as a condition of sustainable development. In addition, the recovery of heavy metals from industrial effluents has increasing attention, as society realizes the necessity for recycling and conservation of essential metals (Doble and Kumar 2005; Dhankhar and Hooda 2011).

Various methods have been used for the removal and recovery of heavy metal ions from aqueous effluents. Among these, flocculation, coagulation, chemical precipitation, electrochemical techniques, ion exchange, membrane-related processes, etc. (Dabrowski et al. 2004; Wang and Chen 2009; Llanos et al. 2010) are the most widely used, and now it has been proved that all have numerous disadvantages. Low selectivity and efficiency performances are special when used for small concentrations of heavy metal ions, the necessity of using expensive chemicals in some methods and the generation of high amount of toxic sludge or high energy consumption are the main disadvantages of these methods (Hamdy 2000; Wang and Chen 2009). Therefore it is necessary to find new alternative methods for the removal of heavy metal ions from aqueous environments that minimize these disadvantages, and this is still an actual scientific issue, with great applicative importance.

Biosorption of heavy metal ions, using cheaper and non-living natural materials of biologic origin has become a promising alternative technology, which can offer an ecologically safer, cheaper and more efficient modality to remove metal ions from industrial wastewater. In comparison with conventional methods, the biosorption can be considered a rapid, reversible, economical and eco-friendly method, which can be used in numerous situations to reduce the environmental pollution. In addition, if the biosorption process is well designed, its efficiency is

very high, resulting in high-quality aqueous effluents after such treatment, which can be reused without problems. However, the cost of the biosorbents is the most important factor in view of the applicability of the biosorption process in wastewater treatment at large scale.

In order to provide the most cheaper biosorbents, for the removal of heavy metal ions from industrial effluents, various kinds of materials of biologic origin (such as agricultural waste and by-products, algae, fungi, bacteria, peat, yeasts, etc.) (Kurniawan et al. 2006; Febrianto et al. 2009; Farooq et al. 2010; Dhankhar and Hooda 2011) have been intensively tested. In the biosorption processes, such materials act as a chemical substrate and the most important advantages of their utilization as biosorbents are: (a) they have a variety of functional groups on their surface, (b) they have a relatively small and uniform distribution of binding sites on the surface, and (c) require only few steps of preparation, because in most cases these materials result from other industrial or/and agricultural activities. All these advantages have permitted the classification of such biosorbents into category of the low-cost materials.

The marine algae fully meets all these advantages, and because they are available in large quantities in many regions and have an excellent retention capacity, their utilization as biosorbents have been successfully tested for the removal of various heavy metal ions (Donmez et al. 1999; Davis et al. 2003; Romera et al. 2007). However, the utilization of marine algae as a feasible alternative for the energy production (Demirbas and Demirbas 2011; Singh et al. 2011), which is advantageous from economical point of view, has determined the increasing quantities of algae biomass to be used for this purpose. Under these conditions the testing of algae wastes that resulted from energy production for the removal of heavy metal ions could be an alternative utilization which offers the possibility to valorize such materials, which are generally discharged or incinerated, having a serious negative impact on environment.

In this chapter are comparatively presented the biosorptive performances of marine algae biomass and of wastes resulted from algae energy production for the removal of various heavy metals ions from aqueous media. In this way, it is ensured a sustainable utilization of marine algae biomass in environmental remediation, in agreement with the principles of sustainable development. The most important characteristics of each kind of material are highlighted in order to anticipate the potential use of these as biosorbents. Also the biosorptive characteristics of marine algae biomass and of its wastes from energy production as a function of experimental procedure and experimental conditions, along with new updates on biosorption process modelling and some recent advanced in mechanism elucidation are outlined. The results presented in this chapter indicates that the algae wastes from energy production have potential to become effective and economical biosorbents for the removal of heavy metals from industrial waste effluents.

2 Bioremediation of Heavy Metal Ions Using Marine Algae Biomass

Biosorption is one of the most innovative and feasible methods to remove heavy metal ions from industrial wastewaters, which use predominantly non-living algae. Although in the literature are few studies that use living algae for the removal of heavy metal ions from aqueous media (Tsezos and Volesky 1981; Lamaia et al. 2005; Chekroun and Baghour 2013), the important drawbacks of this procedure (such as: low and limited biosorption capacity, poison of algae by heavy metal ions, large variations of biosorptive performances as a function of the growth phase of algae, etc.) have determined the shift of interest to biosorption (Lesmana et al. 2009). In case of biosorption are used non-living algae, which are easier and cheaper to obtained, making the biosorption an economical and effective method to remove the heavy metals from wastewater, and consequently determine the potential remediation capacity using algae biomass.

The biosorption capacity of marine algae biomass depends on several important factors, such as: nature and number of functional groups from algae biomass surface, nature of metal ions from aqueous media, and last but not least by the experimental conditions selected for the biosorption process. Understanding how these factors influence the development of biosorption process will permit the design of high efficient treatment system of industrial wastewaters, and from this reason their discussion must be done.

2.1 Marine Algae Biosorbents

In general, marine algae biomass used as biosorbents for the removal of heavy metals ions are obtained by following few elementary steps, as

(i) collection of marine algae from their natural environment (seas waters);
(ii) washing several times with distilled water to remove the impurities;
(iii) drying in air at temperatures lower than 70 °C (to not damage the algae leaves);
(iv) crushing and sieving until to a given granulation (usually lower than 1 mm).

The obtained biomass is recommended to be stored in desiccators (to keep a constant humidity) and is added to the aqueous media which contains heavy metal ions. Because, the marine algae biomasses are easily obtained and require only few numbers of operations, they can be included in the category of low-cost biosorbents.

Heavy metal ions biosorption onto marine algae biomass has been attributed to the presence of some constituents, such as polysaccharides, proteins or lipids from the cell walls surface that contains different types of functional groups (hydroxyl, carboxyl, amino, sulphate, phosphate, etc.) (Karthikeyan et al. 2007; Gupta and

Rastogi 2008). These constituents are present in the structure of all kinds of marine algae (whether they are brown, red or green algae), and their functional groups play the role of active binding sites for heavy metal ions from aqueous media. The nature of functional groups from marine algae surface has been easy highlighted by FI-IR spectra (Lupea et al. 2012; El-Nerm et al. 2015), and prove the algae biomass will act as a chemical substrate and will bond heavy metal ions from aqueous solutions by characteristic chemical (electrostatic and/or complexation) interactions.

However, the uptake of heavy metal ions from aqueous media significantly depends on the geometrical availability of such superficial functional groups, in consequence the preparation steps will have an important role in the determining of biosorption performances.

2.2 *Influence of Operating Parameters on Heavy Metal Ions Biosorption*

Most of studies from the literature have indicated that biosorption of metal ions onto various low-cost materials of biologic origin occurs with maximum efficiency only in well-defined experimental conditions (Donmez et al. 1999; Febrianto et al. 2009; Robals et al. 2016). These conditions are usually established through batch experiments and aim to study the influence of most important operating parameters on the biosorption process efficiency. Such information are important in the design of adequate wastewater treatment system.

The operating parameters that have a significant influence on the biosorption of heavy metal ion onto marine algae biomass are: initial solution pH, biosorbent dose, contact time, initial metal ions concentration and temperature, and their influence on the heavy metal ions biosorption will permit the finding of optimal values.

(a) ***The influence of initial solution pH***

Initial solution pH is one of the most important operating parameters that affect the biosorption capacity of marine algae biomass for heavy metal ions (Mehta et al. 2002; Rangsayatorn et al. 2002; Han et al. 2006). This is because initial pH solution significantly influences not only the speciation and solubility of metal ions from aqueous solution (free ions or complex species), but also the dissociation degree of functional groups from marine algae biomass surface, considered as biosorption sites (Marques et al. 2000; Esposito et al. 2002).

For a given initial concentration, the heavy metal ions remains as free ions in aqueous solution in relatively large pH intervals (acid to neutral media), and their biosorption occurs without any problems. Unfortunately, high pH values (from weak basic media) may cause the precipitation of metal species. The apparition of precipitation drastically influenced the efficiency of metal ions biosorption and in consequence it should be avoided.

More important is the influence of initial solution pH on the dissociation degree of functional groups from marine algae biomass surface. Therefore, in acid media most of functional groups from are protonated, due to competition between protons and heavy metal ions for the active sites from biosorbent surface (Gardea-Torresdey et al. 1990). This makes the values of biosorption capacity to be low, regardless of the type of metal ions or of marine algae biomass used as biosorbent. The increase in the initial solution pH will determine the increase of dissociation degree of functional groups from marine algae biomass surface. In consequence, the number of electrostatic interactions between metal ions and superficial functional groups will increase and this will be determined by the increase of the biosorption capacities values. In Table 1 are summarized the pK_a values of some functional groups that are relatively abundant in most marine algae biomasses and that have the largest importance in the heavy metal biosorption process.

Therefore, finding the optimal pH for maximum biosorption efficiency is very important step, which must be done experimentally for each studied biosorption system (metal ion and marine algae), because this parameter is correlated with the biomass surface charge, ionization degree and metal ions speciation. In most of cases, the biosorption of heavy metal ions from aqueous media onto marine algae biomass occurs with maximum efficiency in a pH range between 4.0 and 6.0 (Table 2), because in this pH domain the predominant specie of most of heavy metals is free ions (M^{2+}) (Marques et al. 2000) and the dissociation degree of superficial functional groups from algae biomass is high, and will facilitate the electrostatic interactions (Donmez et al. 1999; Sari and Tuzen 2008).

When the heavy metal ions exist in aqueous solution as negative charged species (oxoanions), the weak acid–neutral pH domain is not anymore suitable for the biosorption. The most notorious is the case of Cr(VI), which as is known that in aqueous solution is as CrO_4^- or $Cr_2O_7^{2-}$ species (Kushwaha et al. 2012), and which exhibits a maximum biosorption efficiency onto marine algae biomass in a much lower pH interval (see Table 2). This behaviour is determined by the fact that such negatively charged species could be retained only by the protonated active sites of the biosorbent, and these are obtained in strong acid media (see Table 1), where the concentration of protons is very high (Kayalvizhi et al. 2015; Hackbarth et al. 2016).

Usually, various mineral acid solutions (HCl, HNO_3, H_2SO_4) (Yaqub et al. 2012; Uzunoğlu et al. 2014) or alkali solutions (NaOH, KOH) (Areco et al. 2012)

Table 1 pKa values of most important functional groups from marine algae biomass structure (Dean 1995)

Functional group	Ligand atom	pKa	Functional group	Ligand atom	pKa
Hydroxyl	O	9.5–13	Amine	N	8–11
Carboxyl	O	1.5–5.0	Secondary amine	N	13.0
Thiol	S	8.2–10.8	Imine	N	11.5–12.5
Sulfonate	O	1.5	Imidazol	N	6.0

Table 2 Optimal values of operating parameters for the removal of some heavy metal ions from aqueous media by biosorption on marine algae biomass

Heavy metal ion	Marine algae biomass	pH	Biosorbent dosage (g/L)	Contact time (min)	Temperature (°C)	Reference
Cd(II)	*Fucus ceranoides*	4.5	2.5	25	25	Herrero et al. (2006)
	Fucus serratus	4.5	2.5	25	25	Herrero et al. (2006)
	Bifurcaria bifurcata	4.5	2.5	>60	25	Lee and Chang (2011)
	Saccorhiza polyschides	4.5	2.5	>60	25	Lee and Chang (2011)
	Ascophyllum nodosum	4.5	2.5	>60	25	Lee and Chang (2011)
	Laminaria ochroleuca	4.5	2.5	>60	25	Lee and Chang (2011)
	Pelvetia caniculata	4.5	2.5	>60	25	Lee and Chang (2011)
	Cystoseira baccata	4.5	2.5	17	25	Lodeiro et al. (2006)
	Ceramium virgatum	5.0	0.1	60	20	Sarı and Tuzen (2008b)
	Ulva lactuca	5.0	0.1	60	20	Sarı and Tuzen (2008a)
		5.0	8.0	30	20	Wang et al. (2009)
Co(II)	*Ptredocladia capillacea*	5.0	10.0	<60	25	Ibrahim (2011)
	Galaxaura oblongata	5.0	10.0	<60	25	Ibrahim (2011)
	Ulva lactuca	5.0	8.0	–	23	Lupea et al. (2012b)
Cr(III)	*Spirogyra spp*	5.0	3.0	45	25	Bishnoi et al. (2007)
	Chlorella sorokiniana	4.0	1.0	15–20	25	Akhtar et al. (2008)
Cr(VI)	*Fucus vesiculosus*	2.0	2.0	120	Ambient temp.	Murphy et al. (2008)
	Fucus spiralis	2.0	2.0	120	Ambient temp.	Murphy et al. (2008)
	Palmaria palmate	2.0	2.0	30	Ambient temp.	Murphy et al. (2008)
	Polysiphonia lanosa	2.0	2.0	30	Ambient temp.	Murphy et al. (2008)
	Ceramium virgatum	1.5	0.1	90	20	Sari and Tuzen (2008c)
	Ulva lactuca	1.0-1.5	3.0	120	25	El-Sikaily et al. (2007)
Cu(II)	*Fucus serratus*	5.5	0.9	350	25	Ahmady-Asbchin et al. (2008)

(continued)

Table 2 (continued)

Heavy metal ion	Marine algae biomass	pH	Biosorbent dosage (g/L)	Contact time (min)	Temperature (°C)	Reference
	Fucus vesiculosus	5.0	1.0	120	23	Mata et al. (2008)
	Sargassum sp	5.5	1.0	30	25	Karthikeyan et al. (2007)
	Fucus spiralis	5.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Ascophyllum nodosum	4.0	1.0	120	Ambient temp.	Romera et al. (2007)
	Asparagopsis armata	5.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Chondrus crispus	4.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Ulva fasciata	5.5	1.0	30	22	Karthikeyan et al. (2007)
	Chaetomorpha linum	5.0	20	120	23	Ajjabi and Chouba (2009)
	Spirogyra spp.	5.0	1.0	30	Ambient temp.	Lee and Chang (2011)
	Cladophora spp.	5.0	1.0	30	Ambient temp.	Lee and Chang (2011)
Pb(II)	*Fucus spiralis*	5.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Ascophyllum nodosum	5.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Fucus vesiculosus	5.0	1.0	120	23	Mata et al. (2008)
	Asparagopsis armata	4.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Chondrus crispus	4.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Cladophora fascicularis	5.0	2.0	30	25	Deng et al. (2007)
	Spirogyra spp.	5.0	0.5	100	20	Lee and Chang (2011)
	Cladophora spp.	5.0	1.0	30	Ambient temp.	Lee and Chang (2011)
	Ulva lactuca	5.0	0.1	60	20	Sari and Tuzen (2008a)
		5.0	8.0	30	22	Bulgariu et al. (2010)
Ni(II)	*Sargassum glaucescens*	6.0	1.0	120	30	Pahlavanzadeh et al. (2010)
	Padina australis	6.0	1.0	120	30	Kalyani et al. (2004)

(continued)

Table 2 (continued)

Heavy metal ion	Marine algae biomass	pH	Biosorbent dosage (g/L)	Contact time (min)	Temperature (°C)	Reference
	Fucus spiralis	6.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Ascophyllum nodosum	6.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Asparagopsis armata	6.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Chondrus crispus	6.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Codium vermilara	6.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Cladophora spp.	5.0	5.0	–	23	Zakhama et al. (2011)
	Ulva lactuca	4.5	2.0	60	30	Zakhama et al. (2011)
Zn(II)	*Fucus spiralis*	6.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Ascophyllum nodosum	6.0	0.5	120	Ambient temp.	Romera et al., (2007)
	Asparagopsis armata	6.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Chondrus crispus	6.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Codium vermilara	6.0	0.5	120	Ambient temp.	Romera et al. (2007)
	Spirogyra insignis	6.0	1.0	120	Ambient temp.	Romera et al. (2007)
	Chaetomorpha linum	5.0	20	120	25	Ajjabi and Chouba (2009)
	Ulva fasciata sp.	5.0	0.1	20	30	Kumar et al. (2006)

are used for adjusting the pH of aqueous solution to the optimal value, before the adding of biosorbent. The choice of the most suitable solution for pH adjustment must be made so as to avoid the apparition of secondary processes (precipitation, complexation, etc.).

(b) ***The influence of biosorbent dosage***

The efficiency of heavy metal ions biosorption from aqueous media is dependent on the algae biomass dose. In general, for a given initial heavy metal ion concentration, the increase of biosorbent dose reduces the amount of heavy metals retained per biosorbent unit (Gokhale et al. 2008; Finocchio et al. 2010), determined by the concentration gradient between biosorbent and metal ions from aqueous media. An explanation for this variation could be the fact that at higher

dose of biosorbent, the ratio metal ion/algae biomass will be lower, and this will negatively affect the efficiency of biosorption (Mehta and Gaur 2005). High dose of biosorbent determines the apparition of shell effect, which is manifested through the protection of functional groups to be occupied by heavy metal ions, and in consequence the amount of metal ion retained per biosorbent unit will decrease (Farooq et al. 2010). These arguments have made that in the heavy metals biosorption process, lower marine algae biomass dosage has to be recommended.

On the other hand, in the establishment of optimal biosorbent dose must be considered the variation of removal percent also. This parameter depends only on the concentration of heavy metal ions from aqueous media, and it is a measure of biosorption yield. Unlike biosorption capacity (q, mg/g), the removal percent (R, %) increases with the increasing of biosorbent dosage, most probably due to the increase of number of available binding sites (Ucun et al. 2003). In Fig. 1 is illustrated a typical variation of these two parameters (q, mg/g and R, %) as a function of biosorbent dosage, obtained for Cd(II) biosorption onto *Ulva lactuca* sp. marine green algae biomass (Lupea et al. 2012a).

From this reason, the selection of optimal biosorbent dosage must be done after a careful analysis of the experimental values obtained for both parameters (q, mg/g and R, %), to ensure a high biosorptive performance of marine algae biomass (high value of q), but also a high efficiency of biosorption process (high value of R).

In Table 2 are summarized the values of optimal biosorbent dosage found for the biosorption of various heavy metal ions onto different marine algae biomasses.

(c) ***The influence of initial heavy metal ions concentration***

The removal of heavy metal ions by biosorption onto marine algae biomass significantly depends on the initial concentration of metal ions from aqueous media. In general, the increase of initial heavy metal ions concentration results on the increase of biosorption capacity of marine algae biomass. This variation is determined to the fact that the increase of initial metal ions concentration provides a large driving force that will overcome all mass transfer resistances between solid

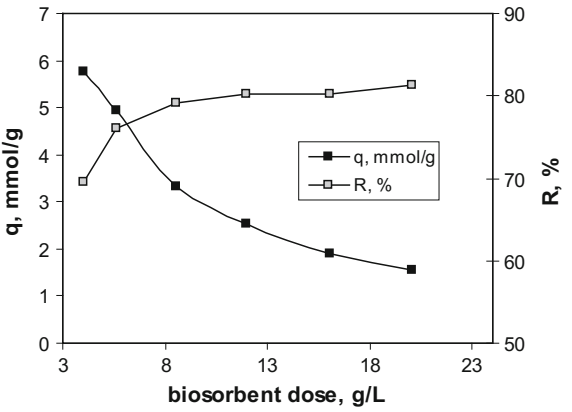

Fig. 1 Influence of biosorbent dosage ob Cd(II) ions biosorption onto *Ulva lactuca* sp. marine green algae biomass

(marine algae biomass) and liquid (aqueous effluents), and in consequence the biosorption of heavy metal ions is higher (Singh et al. 2010). This phenomenon could be used to increase biosorption capacity. For example, Badescu and colab. (Badescu et al. 2015) have reported that the increase of initial Cu(II) concentration from 15 to 210 mg/L determined the increasing of biosorption capacity over 18 times, from 1.12 to 20.28 mg Cu(II)/g dry biomass of *Ulva lactuca* sp. marine green algae. However, it should be noted that this variation is not a linear one. Usually, at very high initial heavy metal ions concentration, the biosorption capacity tends to reach a plateau, which indicates the saturation of biosorbent. For exemplification, in Fig. 2 is illustrated the variation of biosorption capacity of *Ulva lactuca* sp. marine green algae biomass as a function of initial Co(II) concentration (Lupea et al. 2012b).

Such variation of biosorption capacity of marine algae biomasses as a function of initial heavy metal ions concentration indicates that in the biosorption process are mainly involved the functional groups from marine algae biomass surface, and that the interactions between these and the metal ions are most probable electrostatic (ion-exchange type). In a schematic representation, it can be assumed that the heavy metal ions from aqueous media will reach close to the function groups of marine algae biosorbents, where they will interact first with the active sites with largest spatial availability. After these active sites are occupied, they will create a steric blockage that limiting the interactions between metal ions and the functional groups from inside of biosorbent (Bulgariu and Bulgariu 2015).

On the other hand, at low initial concentration of heavy metal ions removal takes place more efficiently than at higher concentrations. This high efficiency is related to the high values of removal percents, which indicate that the concentration of heavy metal ions in effluents treated through biosorption is low (even lower than the values corresponding to the maximum permissible limits (NTPA 002/2005)). Unfortunately, the initial heavy metal concentration interval, where algae waste biomass can be considered an effective biosorbent in the treatment processes of wastewaters, is relatively narrow (>20 mg/L). The increase of the initial

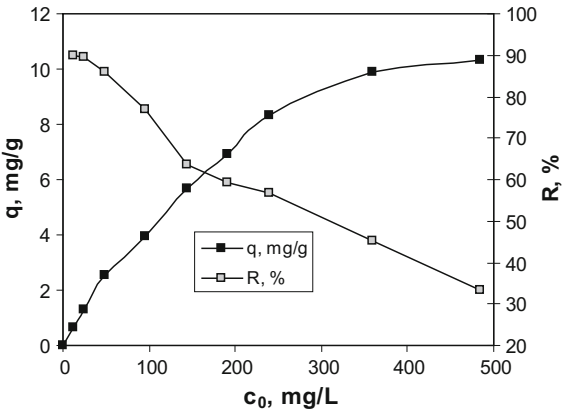

Fig. 2 Influence of initial metal ions concentration on Co(II) biosorption on *Ulva lactuca* sp. marine green algae biomass

concentration of heavy metals over this concentration range results in the increasing of their concentration in effluent solution, and this makes the treatment of wastewaters to be necessary two or more biosorption steps.

(d) ***The influence of contact time***

Heavy metal ion biosorption, using marine algae biomasses as biosorbent, is highly dependent on contact time, and in consequence this parameter should be also optimized. An unsatisfactory value of contact time drastically limits the practical applicability of the biosorption processes, even if the efficiency in the heavy metals removal is high.

Generally, biosorption processes takes place in two subsequent stages: a very fast stage at the beginning, where the amount of heavy metal ions retained increase rapidly within the first minutes (20–60 min), followed by a much slower gradual biosorption, near to the equilibrium. In most of the biosorption processes using marine algae biomasses as biosorbents, no further significant biosorption is noted after 3 h of contact time (Volesky 1987). Therefore, any value of contact time from the second stage can be selected as optimal for a given biosorption system, because here the variation of heavy metal ions biosorption is small. In Table 2 are presented the values of contact time required for the efficient biosorption of heavy metal ions from aqueous media, using different types of marine algae biomasses. It can be observed that the value of optimal contact time is not higher than 180 min in these biosorption processes.

Such two-steps biosorption processes have been reported in many studies from the literature for the retention of heavy metal ions on various low-cost materials (Ho et al. 1996; Gerente et al. 2007; Febrianto et al. 2009; Montazer-Rahmati et al. 2011). The rapid initial biosorption step is generally the result of the fast transfer of metal ions to the surface on the biosorbent particles, while the subsequent slow biosorption process is a consequence of the slow diffusion on metal ions into the pores of the biosorbent particles (Qin et al. 2006). The very fast biosorption of heavy metal ions on marine algae biomass is important from practical point of view, because this will facilitate the scale-up of the biosorption process to smaller reactor volumes, which will ensure efficiency and economy (Liu et al. 2006).

(e) ***The influence of temperature***

The influence of temperature on biosorption efficiency is different as function of the nature of heavy metal ion from aqueous media and type of marine algae biomass used as biosorbent (Monteiro et al. 2010). This parameter is generally used to characterize the biosorption process from thermodynamic point of view, and its optimal value for a given biosorption system must be established experimentally.

Many studies from the literature indicate that the increase of temperature determined the increase of biosorption capacity of marine algae biomass (Gupta et al. 2010; Monteiro et al. 2010; Johansson et al. 2016). Even if the experimental studies have been performed in a relatively narrow temperature range (up to 50 °C, in order to avoid the degradation of algae biomass (Montazer-Rahmati et al. 2011)),

such variation of biosorption capacity with temperature indicates that the biosorption process is endothermic one. The main reasons for the increasing of heavy metal ions biosorption with the increase of temperature can be: (i) an increased number of active sites involved in the biosorption process; (ii) an increased availability of active sites from biosorbent surface to interact with heavy metal ions from aqueous media (Mehta and Gaur 2005); (iii) a decrease of mass transfer resistance in the diffusion layer (Meena et al. 2005), and (iv) an increased stability of complex formed between metal ions and superficial functional groups of algae biomass.

The increase of temperature does not always significantly increase the biosorption capacities of marine algae biomass, indicating that temperature has no significant influence on the metal ions biosorption onto marine algae biomass (Martins et al. 2004; Lodeiro et al. 2006). Thus, in the study performed by Lupea et al. (2012a), it has shown that the increase of temperature with 30 °C determine an insignificant variation of biosorption capacity (0.06 mmol/g) of *Ulva lacutca* sp. marine green algae biomass for Cd(II) ions.

In other studies, it was shown that the biosorption of the same Cd(II) ions increase with the increasing of temperature when *Sargassum* sp. or *Ceramium virgatum* are used as biosorbents, because of the exothermic nature of removal process (Cruz et al. 2004; Sari and Tuzen 2008).

In Table 2 is summarized the optimal values of temperature required for the efficient biosorption of heavy metal ions from aqueous media, using different types of marine algae biosorbents. However, analyzing the increase of biosorption efficiency and the costs of temperature increasing, it is recommended that the removal of heavy metal ion from aqueous solutions by biosorption on marine algae biomass is to be performed at ambient temperature, especially by economical considerations (Wang 2002).

Each of these parameters must be analyzed in the experimental studies of heavy metal ions removal by biosorption onto marine algae biomasses, and the obtained results will allow the finding of the optimal conditions that will ensure maximum efficiency of the biosorption process.

2.3 *Modelling of Biosorption Process of Heavy Metals on Marine Algae Biomass*

In order to understand the biosorption mechanism and to characterize the heavy metals biosorption process onto marine algae biomass, it is necessary to analyze the experimental data both from equilibrium and kinetics point of view. The information obtained from biosorption modelling are very useful for the design of treatment systems of wastewater with large-scale applications.

Therefore, in order to make the biosorption process useful for practical applications it is necessary his mathematical description, and this can be done through

modelling. The modelling of biosorption processes implies both the modelling of biosorption isotherms (which represent the equilibrium distribution of the studied heavy metal ions between the phases of the solid biosorbent and aqueous solution) and kinetic modelling (necessary to identify the biosorption mechanism). Practically, the modelling of biosorption process means analyze of the experimental data using various isotherms and kinetics models, and the calculation of characteristic parameters. The selection of the best-fit model is done based on the values of correlation coefficients (R^2), obtained from linear or nonlinear regression.

In case of marine algae biosorbents, the isotherms are usually described with Langmuir and Freundlich models (due to the shape of biosorption isotherms), while the kinetics of biosorption is more frequently analyzed with pseudo-first-order- or pseudo-second-order kinetics models (Chojnacka 2010). The mathematical equations of these models are presented in Table 3.

Although in the literature are presented numerous other models that can be used for modelling, the large applicability of these is determined by their usefulness in the description of heavy metal ions biosorption using marine algae biomass. Thus, the Langmuir model considers that biosorption occurs on homogeneous surface until a complete monolayer coverage is formed at the surface of biosorbent, while the Freundlich model assumes that biosorption takes place on heterogeneous surface and it is not restricted to the formation of a monolayer (Chong and Volesky

Table 3 Mathematical equation of isotherm and kinetics models (Ho 2006; Gerente et al. 2007; Wang and Chen 2009; Febrianto et al. 2009; Montazer-Rahmati et al. 2011)

Model	Mathematical equation	Notations
Isotherm models		
Freundlich	$\ln q_e = \ln K_F + \frac{1}{n} \ln c_e$	q_e is the equilibrium metal biosorption capacity; c_e is the equilibrium metal ions concentration in solution; K_F is a biosorption equilibrium constant, representative of the biosorption capacity; n is a constant related with the biosorption intensity
Langmuir	$q_e = q_{\max} \cdot \frac{K_L \cdot c_e}{1 + K_L \cdot c_e}$	q_{max} is the maximum adsorption capacity upon complete saturation of biosorbent surface; K_L is Langmuir constant, related to the biosorption/desorption energy.
Kinetics models		
Pseudo-first model	$\ln(q_e - q_t) = \ln q_e - k_1 \cdot t$	q_e, q_t are the amounts of heavy metals retained on weight unit of biosorbent at equilibrium and at time t, (mg/g); k_1 is the rate constant of pseudo-first order kinetics equation, (min^{-1})
Pseudo-second model	$\frac{t}{q_t} = \frac{1}{k_2 \cdot q_e^2} + \frac{t}{q_e}$	q_e, q_t are the amounts of heavy metals retained on weight unit of biosorbent at equilibrium and at time t, (mg/g); k_2 is the rate constant of pseudo-second order kinetics equation, (g/mg · min)

1995; Rangabhashiyam et al. 2014). Even if the basic assumptions of these models were not fulfilled due to the heterogeneity of the biosorbent surface, they were quite successful in predicting the practical biosorption capacity and optimization of the biosorption system design.

The shape of experimental biosorption isotherms obtained for most removal processes of various heavy metal ions using different types of marine algae biomass is a first indication that these biosorbents have a finite number of available biosorption sites, and in consequence the Langmuir model was found the be best best-fit isotherm model, regardless of experimental conditions. The very well concordance between experimental results and Langmuir model show that the marine algae biosorbents have a surface quite homogeneous and that the biosorption of heavy metal ions from aqueous solution occurs until the formation of monolayer coverage on the outer surface of biomass (Rangabhashiyam et al. 2014). So, it is possible to calculate the maximum biosorption capacity (q_{max}, mg/g) of such biosorbents for a given metal ion in defined experimental conditions, and this could be a measure of biosorptive performances of marine algae biomasses in heavy metals removal processes. In Table 4 are summarized the values of Langmuir model parameters obtained in case of heavy metal ions biosorption on various marine algae biomasses.

Also, the values of Langmuir constant (K_L, L/g) (Table 4), which are related to the biosorption energy (Rangabhashiyam et al. 2014), shows that in most of cases between superficial functional groups of marine algae biomasses and heavy metal ions from aqueous solution occurs strong interaction, most probably of ion-exchange type.

The analysis of experimental date with Langmuir isotherm model indicates that during of biosorption process, the heavy metal ions will interact with the functional groups from out surface of marine algae biomass, predominantly by chemical forces, until entire surface of biosorbent particle will be covered with metal ions. After the coverage of biosorbent particle is complete, the biosorption process does not take place anymore, and the functional groups that could still interact with the metal ions becomes geometrical unavailable.

In comparison with Langmuir model, Freundlich model has a more limited applicability in the mathematical description of heavy metal ions biosorption onto marine algae biomass. This is because the Freundlich isotherm model can be used for the modelling of biosorption processes that occurs on heterogeneous surface or surface supporting sites of different affinity (Farooq et al. 2010), characteristics which are not specific to the marine algae biosorbents. However, in some cases the biosorption of heavy metal ions on marine algae biosorbents are better described by Freundlich isotherm model and several examples are presented in Table 5.

This does not necessarily mean that some marine algae biomasses have a more heterogeneous surface in comparison with other. Most probably in such cases, on the algae biomass surface are present several types of binding sites which have different affinity for heavy metal ions from aqueous solution. Therefore during of biosorption, the metal ions will interact first with the binding sites which have the

Table 4 Langmuir parameters for the removal of some heavy metal ions on marine algae biomass

Heavy metal ion	Marine algae biomass	R^2	q_{max} (mg/g)	K_L (L/mg)	Reference
Cd(II)	*Laminaria* sp.	0.972	104.53	0.01	Delle (2001)
	Oedogonium sp	0.995	88.20	0.02	Gupta and Rastogi (2008)
	Lobophora variegata	0.982	167.91	0.15	Jha et al. (2009)
	Pelvetia caniculata	0.992	75.00	0.07	Lodeiro et al. (2005)
	Cystoseira baccata	0.980	77.56	0.09	Lodeiro et al. (2006)
	Gelidium	0.998	18.0	0.19	Vilar et al. (2006)
	Ulva lactuca	0.988	41.66	0.003	Lupea et al. (2012a)
Cu(II)	*Cladophora* sp.	0.961	47.02	0.14	Deng et al. (2007)
	Fucus serratus	0.993	101.73	0.26	Ahmady-Asbchin et al. (2008)
	Undaria pinnatifida	0.965	78.88	0.06	Chen et al. (2008)
	Ulva fasciata sp.	0.999	26.88	0.25	Kumar et al. (2006)
	Spirogyra sp.	0.995	38.61	0.04	Lee and Chang (2011)
	Laminaria sp.	0.990	61.59	0.02	Liu et al. (2009)
Ni(II)	*Undaria pinnatifida*	0.984	28.89	0.10	Chen et al. (2008)
	Oedogonium sp.	0.985	40.98	0.03	Gupta et al. (2010)
	Laminaria sp.	0.954	66.33	0.02	Liu et al. (2009)
	Cystoseria indica	0.999	47.62	0.03	Pahlavanzadeh et al. (2010)
	Nizmuddinia zanardini	0.999	50.00	0.02	
	Sargassum glaucescens	0.998	52.63	0.02	
Pb(II)	*Cladophora* sp.	0.998	200.42	0.04	Deng et al. (2007)
	Oedogonium sp.	0.998	144.92	0.02	Gupta and Rastogi (2008)
	Nostoc sp	0.990	93.46	0.02	Gupta and Rastogi (2008)
	Lobophora sp.	0.974	580.16	0.11	Jha et al. (2009)
	Cystoseira baccata	0.980	182.33	0.37	Lodeiro et al. (2006)
Zn(II)	*Ulva fasciata* sp.	0.998	13.50	0.09	Kumar et al. (2007)
	Laminaria sp.	0.983	54.26	0.01	Liu et al. (2009)

higher affinity, after that the binding strength decreases with the increase degree of occupation (Febrianto et al. 2009).

But in most of cases, the Freundlich isotherm model is used to estimate the biosorption intensity of heavy metal ions towards a given biosorbent, through n constant (see Table 3). A favourable biosorption process tends to have Freundlich constant n, between 1 and 10 (Delle 2001). Larger value of n (smaller value for 1/n)

Table 5 Freundlich parameters for the removal of some heavy metal ions on marine algae biomass

Heavy metal ion	Marine algae biomass	R^2	n	K_F (mmol g^{-1}) (L $mmol^{-1})^{1/n}$	Reference
Cd(II)	*Laminaria* sp.	0.847	4.98	64.07	Liu et al. (2009)
	Oedogonium sp	0.934	1.63	4.89	Gupta and Rastogi (2008)
	Lobophora variegata	0.906	1.27	17.02	Jha et al. (2009)
	Pelvetia caniculata	0.941	2.80	13.03	Lodeiro et al. (2005)
	Cystoseira baccata	0.970	3.10	75.31	Lodeiro et al. (2006)
	Ulva lactuca	0.910	1.43	0.56	Lupea et al. (2012a)
Cu(II)	*Cladophora* sp.	0.998	2.45	84.38	Deng et al. (2007)
	Fucus serratus	0.978	2.83	161.54	Ahmady-Asbchin et al. (2008)
	Undaria pinnatifida	0.947	1.52	6.65	Chen et al. (2008)
	Ulva fasciata sp.	0.960	0.45	2.22	Kumar et al. (2006)
	Spirogyra sp.	0.958	4.15	9.67	Lee and Chang (2011)
Ni(II)	*Undaria pinnatifida*	0.941	2.52	7.17	Chen et al. (2008)
	Laminaria sp.	0.845	3.78	36.39	Liu et al. (2009)
	Cystoseria indica	0.957	1.77	2.75	Pahlavanzadeh et al. (2010)
	Nizmuddinia zanardini	0.973	1.66	2.09	
	Sargassum glaucescens	0.974	1.63	2.04	
	Padina australis	0.969	1.72	1.08	
Pb(II)	*Cladophora* sp.	0.997	3.62	163.47	Deng et al. (2007)
	Oedogonium sp.	0.925	1.75	8.07	Gupta and Rastogi (2008)
	Nostoc sp	0.949	2.28	8.34	Gupta and Rastogi (2008)
	Lobophora sp.	0.928	1.03	93.24	Jha et al. (2009)
	Cystoseira baccata	0.840	6.00	153.33	Lodeiro et al. (2006)
Zn(II)	*Ulva fasciata* sp.	0.983	0.44	1.42	Kumar et al. (2007)
	Laminaria sp.	0.921	2.92	23.53	Liu et al. (2009)

indicates strong interactions between marine algae biomass and heavy metal ions (see Table 5), while a value of n equal to 1, suggests linear biosorption process, leading to identical biosorption energy for all sites.

Beside the equilibrium modelling, which is very important to characterize the efficiency of biosorption processes, the kinetic modelling it is also necessary to describe the biosorption mechanism. In order to study the biosorption mechanism and its potential rate-controlling step (which can include chemical interactions or mass transport), various kinetic models have been used for the analysis of experimental data. In addition, information on the kinetics of metal ions biosorption on a given biosorbent is necessary in order to obtain optimum operating conditions for industrial-scale metal removal processes (Chojnacka 2010).

It is well known that biosorption is a relatively quick process, in which the equilibrium stage is in most of cases reached within few minutes. The high rate of biosorption makes the selection of proper kinetic model in some cases to be difficult. However, the biosorption processes of heavy metal ions on marine algae biomasses are usually described using pseudo-first-order- and pseudo-second-order kinetics models. The main difference between these two models is that the pseudo-first-order kinetic model assumes that the binding of metal ions from aqueous solution on biosorbent surface is done on only one biosorption site (Eq. (1)):

$$R - O^{-}_{(solid)} + M^{2+}_{(aq)} \rightarrow R - O^{-} M^{2+}_{(solid)} \quad (1)$$

while in case of pseudo-second-order kinetic model, the metal ions are bonded on two binding sites from the biosorbent surface (Eq. (2))

$$2\,R - O^{-}_{(solid)} + M^{2+}_{(aq)} \rightarrow (2\,R - O^{-}) M^{2+}_{(solid)} \quad (2)$$

where: $R–O^{-}$ is the biosorbent skeleton.

The mathematical equations of these are also presented in Table 3. Based of these linear equations, the fitting of experimental data will allow the determination of biosorption limiting step order and rate constant, which are the characteristic parameters in kinetics modelling. The selection of most suitable kinetic models is done also using the values of correlation coefficients (R^2), obtained from linear or nonlinear regression.

In Table 6 are summarized the values of kinetic parameters of the pseudo-first- and pseudo-second-order kinetic models obtained for the biosorption of some heavy metal ions on various types of marine algae biomasses.

The order of biosorption limiting step is related to the mechanism of biosorption, and in most of biosorption processes that use marine algae biomass the removal of heavy metal ions from aqueous solutions are very well described by the pseudo-second-order kinetic model. According to this model, in the biosorption process the rate limiting step is the chemical interaction (most frequently ion exchange or surface precipitation) between superficial functional groups of biosorbent and heavy metal ions from aqueous solution, which involve valent forces by sharing or exchange of electrons (Ho et al. 1996; Chojnacka 2010). Also, the high values of rate constants (k_2, $g/mg \cdot min$) obtained in mentioned biosorption

Table 6 Kinetics parameters for the pseudo-second order model obtained in case of some heavy metal ions biosorption on marine algae biomass

Heavy metal ion	Biosorbent	Kinetic parameters			References
		R^2	q_e (mg/g)	k_2 (g/mg · min)	
Cd(II)	*Laminaria hyperborea*	0.997	31.3	0.024	Freitas et al. (2008)
	Sargassum muticum	0.997	38.4	0.004	Freitas et al. (2008)
	Lobophora variegata	0.995	94.2	0.006	Jha et al. (2009)
	Cystoseira baccata	0.999	56.2	0.007	Lodeiro et al. (2006)
	Fucus vesiculosus	0.999	52.8	0.051	Mata et al. (2008)
	Ceramium virgatum	0.999	116.9	0.003	Sarı and Tuzen (2008b)
Cu(II)	*Ulva fasciata*	0.999	9.7	0.013	Karthikeyan et al. (2007)
	Sargassum sp.	0.999	9.6	0.010	
	Fucus vesiculosus	0.999	41.9	0.002	Mata et al. (2008)
Pb(II)	*Laminaria hyperborea*	0.996	50.3	0.020	Freitas et al. (2008)
	Sargassum muticum	0.997	38.2	0.017	Freitas et al. (2008)
	Oedogonium sp.	0.996	117.6	0.064	Gupta and Rastogi (2008)
	Nostoc sp.	0.992	89.3	0.038	Gupta and Rastogi (2008)
	Lobophora variegata	0.967	229.5	0.010	Jha et al. (2009)
	Cystoseira baccata	0.999	142.9	0.001	Lodeiro et al. (2006)
	Fucus vesiculosus	0.999	102.2	0.009	Mata et al. (2008)
Zn(II)	*Laminaria hyperborea*	0.991	19.2	0.075	Freitas et al. (2008)
	Sargassum muticum	0.995	34.1	0.012	Freitas et al. (2008)
	Ulva fasciata	0.976	3.2	0.168	Kumar et al. (2007)

systems (Table 6) show that the rate of the biosorption process is limited by the availability of heavy metal ions and functional groups from biosorbent surface to interact. When the availability of the superficial functional groups is higher, the rate of adsorption process is also higher, and the equilibrium stage is attained very quickly.

The very well concordance between experimental data and pseudo-second kinetic model is reported in the literature for various types of adsorbent materials of biologic origin (Wang and Chen 2009; Febrianto et al. 2009; Montazer-Rahmati et al. 2011), and the main advantage of this model is its high accuracy in describing the whole kinetic experimental data.

2.4 Overview on Marine Algae Biosorbents

As it was discussed previously, the biosorption processes which used marine algae biomass as biosorbents is a very useful tool in the treatment of aqueous effluents contaminated with metal ions, mainly due to their advantages such as economic and technologic viability, quantitative release of retained metal ions, minimization of secondary waste, easy of operating, high efficiency, low cost of biosorbents preparation, etc. (Romera et al. 2007; Wang and Chen 2009).

Although, such biosorption studies have been performed still from the middle of last century (Tuhy et al. 2014) this skilful method has found no industrial applications at this moment, this method has found no industrial applications at this moment. This is because the utilization of marine algae biomass for the heavy metal ions biosorption has several drawbacks which must be still solved. The most important of these are

(i) ***the biosorption capacity of marine algae biomass for various heavy metal ions, is in most of cases lower that the ion exchange resins***. In order to solve this problem, the researches have been directed to the utilization of various physical and chemical treatments of marine algae biomass that changes the algae surface properties and that provide additional binding sites for the heavy metal ion uptake. Thus, the treatments such as heating/boiling, freezing, crushing, chemical treatments with various reagents (mineral acids, alkali, common inorganic salts, organic compounds), etc., have followed to enhance the metal ions biosorption on marine algae biomass (Ebrahimi et al. 2009, Bulgariu and Bulgariu 2014). Such treatments act to the surface of cell walls membrane and in most of cases provides a greater number of active sites, which determine the increase the biosorption capacity. Unfortunately, the increase of biosorption capacity of marine algae biomass after such treatments is not always a spectacular one, especially considering that the enhancement of biosorption capacity must done without using additional expensive additives, and so the cost of biosorbent preparation remains low (Bulgariu and Bulgariu 2014).

(ii) ***the marine algae biomasses have low mechanical resistance and short life of utilization***. Thus, the easiest technological operations (as pumping or mixing) can destroy the fragile structure of marine algae biomass, which will drastically influence the efficiency of biosorption process. On the other hand, the same mechanically fragile structure of marine algae biomass is also

responsible by the short life of utilization of these biosorbents, because their regeneration and reuse is sometimes difficult to achieve. In order to overcome this inconvenient, many studies from the literature have reported the possibility to immobilize marine algae biomasses on various supports. Various natural polymer (such as agar or alginate) or synthetic compounds (such as silica gel or polyacrylamide) have been used as supporting materials for the immobilization of marine algae biomass (Bayramoğlu et al. 2006; Montazer-Rahmati et al. 2011). The natural polymers are often preferred to synthetic polymers due to non-toxicity on biomass, but the use of this procedure makes that some functional groups from algae biomass surface to become unavailable for metal ions from aqueous solution, because they are blocked by interactions with the polymer matrix. Therefore, even if the mechanically resistance of such immobilized marine algae biomass is significantly improved, their efficiency in biosorption processes of heavy metal ions is often lower (Singh et al. 2011).

(iii) ***the utilization of marine algae biomass in the treatment of wastewater may cause secondary pollution of aqueous effluents***. The secondary pollution appears due to the dissolving of some organic compounds from the biomass structure during biosorption and it is responsible for the increasing of oxygen demand (CCO index) of treated effluent, which is also undesirable because affects its quality. A solution for this problem could be the use of marine algae biomass; first to obtain the oil necessary for the production of biofuels, and then as biosorbent to remove the heavy metal ions from aqueous media. In this way, the easily soluble organic compounds from biomass structure are eliminated since the extraction step, and the resulting material will act as a chemical substrate of biological origin, where superficial functional groups are strongly bonded to the biomass skeleton (Bulgariu and Bulgariu 2012, 2013).

The careful analysis of these drawbacks makes necessary the re-evaluation of technological aspects related to biosorption processes which use marine algae biomass as biosorbent for the removal of heavy metal ions, in order to highlight their feasibility for practical applications.

3 Bioremediation of Heavy Metal Ions Using Marine Algae Waste Biomass

In the last years, the necessity to cover the fuel market requirement has made that large quantity of marine algae biomass to be used for production of biofuels (Singh et al. 2011; Halim et al. 2011). In addition, the extraction of oil from marine algae biomass for biofuels production has proved to be more economical efficient than the removal of heavy metals from industrial wastewater. After oil extraction step, the remaining algae biomass is considered a waste and it is usually discharged or

incinerated, becoming a serious problem for environmental protection (Sims et al. 2010). Until now, only few studies from literature have shown that this waste biomass can be used as low-cost biosorbent for the removal of heavy metals from aqueous media (Bulgariu and Bulgariu 2013, 2016; Xie et al. 2014), even if for such biosorbents the effect of secondary pollution is drastically minimized.

3.1 Marine Algae Waste Biosorbent

In the most general way, the algae waste biomasses are obtained from algae biomass, after solvent extraction operations, when certain types of components (such as lipids, fatty acids, other compounds) are extracted from algae biomass structure (Bulgariu and Bulgariu 2015). The extraction step is performed with common organic solvents (most frequently used being n-hexane or benzene) in Soxhlet extractors and the analysis of the obtained waste biomass has show that

(i) the content of C, O, S and P slightly decreased, suggesting that only certain components from the biomass composition were removed through solvent extraction.

(ii) the specific surface area significantly increase after extraction step, mainly due to the disruption of walls cells of marine algae. Thus, after oil extraction with n-hexane an increase of BET surface area from 0.56 m^2/g to 1.71 m^2/g in case of *Chlorella vulgaris* algae (Xie et al. 2014), and from 0.63 m^2/g to 1.28 m^2/g in case of *Ulva lactuca* algae (Bulgariu and Bulgariu 2015) is reported in the literature. The scanning electron microscopy images presented in these studies confirm that after extraction step, the surface of marine algae waste biomass becomes more irregular, and probably these irregularities are responsible by the increase of specific surface area of biosorbent.

Due to the presence of these irregularities the mass transfer resistance inside of pores decrease, which will facilitate the metal ions diffusion, and thus will contributing to the improvement of biosorption process efficiency (Yeh and Chang 2012).

(iii) IR spectra indicate that most functional groups from marine algae biomass structure are not affected by the extraction step, and most of absorption peaks from FT-IR spectra remains unchanged. Thus, in case of *Ulva lactuca* marine green algae after oil extraction with n-hexane, (Fig. 3), only the absorption bands corresponding to the easy extractable compounds (such as lipids, cerides, etc.) disappear or decrease in intensity. All others functional groups (such as: hydroxyl, amine, carbonyl, carboxylic groups, etc.) from polysaccharides and proteins remain in the structure of waste biomass, and these may interact with heavy metals during of biosorption process.

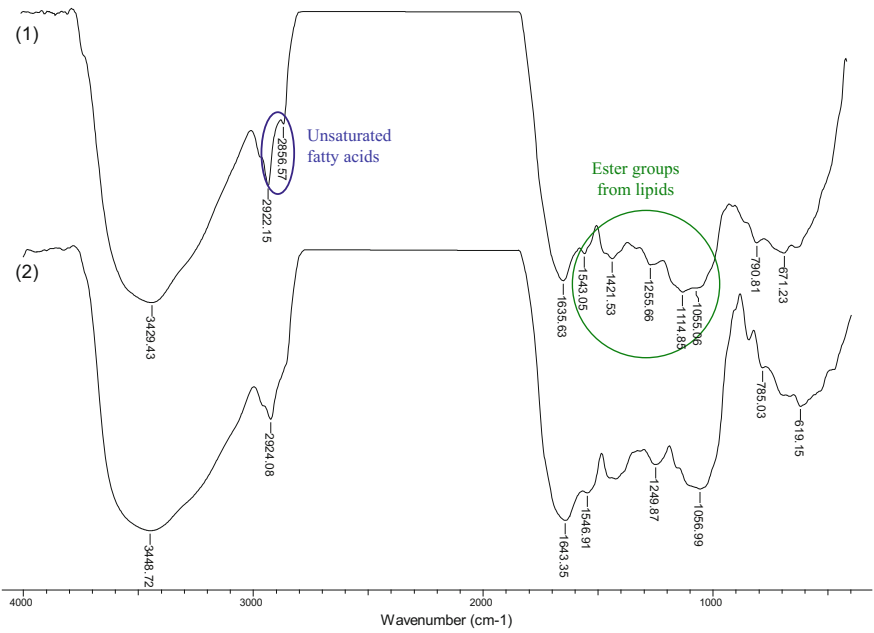

Fig. 3 IR spectra of *Ulva lactuca* sp. marine green algae biomass before (**1**) and after (**2**) extraction of oil with n-hexane

On the basis of these observations it can be said that in case of marine algae biomass, the nature of functional groups responsible for the heavy metal ions uptake is not significantly changed, but their number is improved mainly due to the disruption of walls cells, during of extraction. After extraction, the obtained waste biomass should be washed to remove impurities, dried in air at a given temperature (50–60 °C) for a determined period of time, crushed and sieved to a given particle size (1.0÷1.5 mm), and stored in desiccators for its use as biosorbent.

3.2 Evaluation of Biosorptive Performances of Marine Algae Waste Biosorbents

Since the nature of functional groups is not changed too much, it is expected that the maximum efficiency of biosorption processes that use marine algae waste biomass as biosorbents are to be obtained in the same optimal conditions (initial solution pH, biosorbent dosage and temperature) as in case of marine algae biomass. Only the initial metal ions concentration and contact time may change significantly, because these operating parameters are related to the number of superficial functional groups. Therefore, in order to evaluate the biosorptive performances of marine algae waste biomass, the influence of initial metal ion concentration and

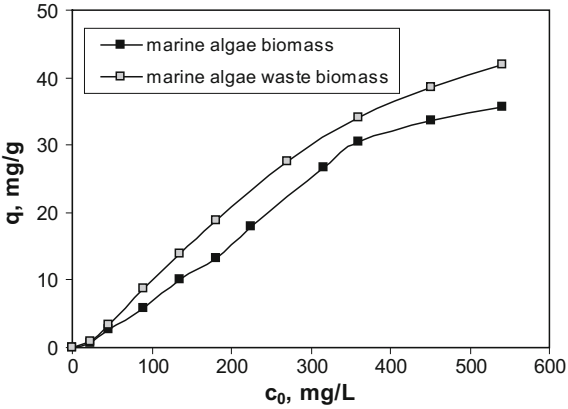

Fig. 4 Influence of initial metal ions concentration on Cd(II) biosorption on *Ulva lactuca* sp. marine green algae biomass, before and after extraction step

equilibrium contact time on the biosorption efficiency must be examined, in comparison with un-extracted marine algae.

In the few studies from literature on this topic (Long et al. 2014; Bulgariu and Bulgariu 2015) it was shown that the marine algae waste biomass has better biosorptive performances than marine algae biomass, and this improvement is more evident at high initial heavy metals concentration. In Fig. 4 is illustrated, for exemplification, the variation of the amount of heavy metals retained on weight unit of biosorbent (q, mg/g) as a function of initial Cd(II) ions concentration, when is used *Ulva lactuca* sp. before (marine algae biomass) and after extraction step (marine algae waste biomass).

These experimental results suggest that in the biosorption mechanism of Cd(II) onto marine algae and marine algae waste biomass are involved, predominantly electrostatic (ion exchange) interactions and the efficiency of biosorption process depends on the availability of functional groups from biosorbent surface. Thus, when the marine algae biomass is subjected to the solvent extraction process, this will break the cell walls of algae, and will spatially activate some functional groups that were inactive before. In consequence, the biosorption capacity of obtained biosorbent will increase. The change of functional groups availability after solvent extraction step have determined a growth of biosorption capacity of *Ulva lactuca* sp. marine algae biomass with 20–30%, for heavy metal ions as Pb(II), Cd(II), Co (II) or Zn(II) from aqueous media (Bulgariu and Bulgariu 2012, 2016), and thus is increased the economical feasibility of this biosorbent.

On the other hand, the equilibrium modelling of obtained isotherms indicate that Langmuir model best describe the experimental results in all cases, indicating the forming of monolayer coverage of the heavy metal ions on the outer surface of the marine algae waste biomass. In Table 7 are summarized the values of maximum biosorption capacity (q_{max}, mg/g) of marine algae biomass and marine algae waste biomass obtained from Langmuir isotherm equation, for the biosorption of some heavy metal ions.

Table 7 Comparative values of maximum biosorption capacity (q_{max}, mg/g) and contact time for the biosorption of some heavy metal ions on marine algae and marine algae waste biomass (Bulgariu and Bulgariu 2012; Lupea et al. 2012a, b)

Metal ion	Marine algae biomass		Marine algae waste biomass	
	q_{max}, (mg/g)	t (min)	q_{max}, (mg/g)	t (min)
Pb(II)	58.13	60	66.30	20
Cd(II)	27.81	60	34.85	40
Co(II)	9.89	60	16.50	20

It can be observed that the values of maximum biosorption capacity (q_{max}, mg/g) were slightly higher in the case of marine algae waste biomass, than in case of marine algae biomassThe increase of this parameter clearly shows that the specific surface area of biosorbent has been increased, and in consequence a high number of metal ions are required to form complete monolayer coverage.

On the other hand, by studying the effect of contact time between biosorbent (marine algae biomass and marine algae waste biomass) and heavy metal ions from aqueous solution, it can be observed that the biosorption efficiency increased along contact time, as is expected. For exemplification, in Fig. 5 is illustrated the influence of contact time on the Pb(II) ions biosorption efficiency, when is used *Ulva lactuca* sp. before (marine algae biomass) and after extraction step (marine algae waste biomass) as biosorbents.

Beside an increase of biosorption efficiency along of contact time, which is normal in case of heavy metal ions biosorption processes, these results have shown another important advantage in the using of marine algae waste biomass as biosorbent, namely that the required time for the biosorption process is significantly lower. Several examples are also summarized in Table 7.

The lower values of contact time necessary to reach the equilibrium state in case of marine algae waste biomass is one more argument which sustains the hypothesis of the biosorption process of heavy metal ions that occurs predominantly by

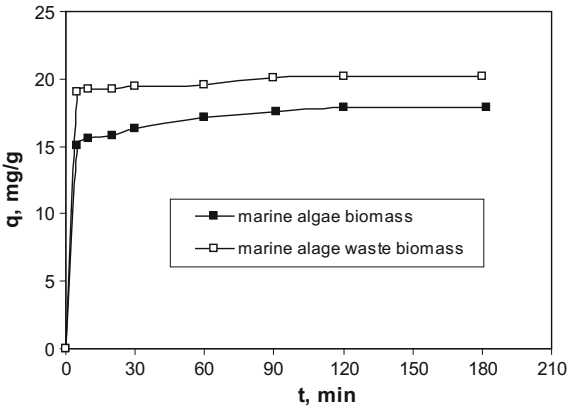

Fig. 5 Influence of contact time on Pb(II) biosorption on *Ulva lactuca* sp. marine green algae biomass, before and after extraction step

electrostatic interactions (between positive charged metal ions and negative charged functional groups), and is influenced by the availability of functional groups to interact. If the availability of superficial functional groups is higher, the rate of biosorption process is also higher. Therefore, when the marine algae biomass is used first in solvent extraction step, the disruption of cell walls makes that the functional groups from resulted biomass to be more available for the interactions with metal ions from aqueous solution. In consequence, the rates of biosorption processes on such biosorbents are higher, even with one order of magnitude, than in case of marine algae biomasses (Bulgariu and Bulgariu 2016).

3.3 Overview on Marine Algae Waste Biosorbents

Although, the utilization of marine algae waste biomass as biosorbent (instead of marine algae biomass) for the biosorptive removal of heavy metal ions from aqueous media significantly reduces the secondary pollution of treated effluents, several technical aspects must be solved before such materials to be used for wastewater treatment, at large scale. The most important are (Bulgariu and Bulgariu 2016)

(i) the removal of the traces of organic solvents from biomass by a simple procedure which does not involve high temperatures (because at high temperatures the biomass structure can be destroyed and the number of functional groups reduced);
(ii) improving of the algae waste performances in biosorption processes (because low biosorption capacities generate large amounts of biomass loaded with heavy metals that is also an environmental problem) (Long et al. 2014);
(iii) prevention of column clogging in continuous systems, which due to the small size of biomass particles (necessary to ensure the high efficiency of oil extraction process), does not allow the proper passage of aqueous solution.

The treatment of marine algae waste biomass with alkaline solutions (ex. 0.1 N NaOH solution) has been proposed in literature (Bulgariu and Bulgariu 2014, 2016) to solve the first two problems. As it is shown in these studies, the alkaline treatment of marine algae waste biomass has two essential roles, namely: (i) reducing the hydrophobicity of the biomass surface, which makes that the traces of organic solvents remained from the extraction step to be eliminated at usual drying temperature (55–60 °C), and usually in a single heat treatment stage, and (ii) improves the dissociation degree of most superficial functional groups, and thus will increase the number of binding sites from the biomass surface (Bulgariu and Bulgariu 2014). These solutions have been chosen so that the cost of obtained biosorbent remains low and without the need of taking supplementary steps for its preparation.

Beside the immobilization of marine algae waste biomass in polymer matrix, which is described in the literature as one of the possibilities that permit the

utilization of such biosorbents in continuous treatment systems, our previous studies (Bulgariu and Bulgariu 2013, 2016) have indicated that the mixing of marine algae waste biomass with a cheap and commercially available anion exchanger resin, such as Purolite A-100, could be also an option. The mixing of marine algae waste biomass with Purolite A-100 resin (considered an inert material with respect of most of heavy metal ions) can be done mechanically, and this procedure has been shown to be effective because it ensures the passing of aqueous solution through the column in a wide flow rate range.

However, the marine algae waste biomasses are still characterized by a low mechanical resistance, and the minimization of this drawback remains a challenge for the further researches, in order to design continuous treatment systems of wastewater contaminated with heavy metal ions.

4 Bioremediation of Heavy Metal Ions Using Marine Algae Biochar

Beside the biofuels production, another procedure that can be used for green energy production and that has gained increased credibility in the last years is the biomass gasification (Heidenreich and Foscolo 2015). Generally, the gasification supposes the transformation of biomass in gases, which may be then used to obtain energy in turbines or boilers (Lan et al. 2015). In case of marine algae biomass or marine algae waste biomass utilization in gasification processes has two major advantages:

(i) the volume of biomass waste is considerable reduced (up to 75%), which represents an important benefit for environment;
(ii) the cost of energy production is lower in comparison with classical combustion methods, because these processes generally occurs at relatively low temperatures (until 500 °C) and in low-oxygen atmosphere (Asadullah 2014; Mohan et al. 2014).

The solid material remained after algae biomass gasification, generally called biochar, is mostly controlled deposited or used for the manufacture of building materials (Agarwal et al. 2015). Nevertheless, the preliminary experimental studies performed by us have shown that such biochar has structural and textural characteristics which recommend it as potential biosorbent in decontamination processes of environment.

Unfortunately because the nature of algae biomass used as feedstock for biochar production, and the thermal treatment conditions (particularly temperature, but also the treatment time it is important), strongly affect the biosorptive characteristics of obtained biochar, it is still a need to undertake detailed studies about the possibility to use this material as biosorbent for heavy metal ions removal (Bird et al. 2011; Maddi et al. 2011).

4.1 Marine Algae Biochar Biosorbent

The changes that occur in the structure of marine algae biomass after its transformation in biochar through thermal treatment can be easy highlighted using IR spectra. The IR spectra recorder in case of *Ulva lactuca* sp. marine algae biomass before and after thermal treatment at 450 °C, illustrated in Fig. 6, clearly shows this.

Thus, after thermal treatment the IR spectra of marine algae biochar (spectra b) indicate that the most adsorption bands are less split, probably due to the breaking of physical bonds between functional groups of marine algae biomass. Also after thermal treatment, the most important functional groups from biomass surface are converted in their reduced form (for example: hydroxyl groups (3429 cm^{-1}) and carbonyl or ether groups (1635 cm^{-1})). Another significant difference is the drastically intensity decrease of the peak from 2922 cm^{-1} that correspond to the C–H stretching vibration of aliphatic hydrocarbons radicals. The significant decrease of this peak shows that after thermal treatment most of aliphatic hydrocarbons chains from marine algae biomass structure were removed by thermal treatment (probably as CO_2 and H_2O), and the obtained biochar contains predominantly aromatic scraps.

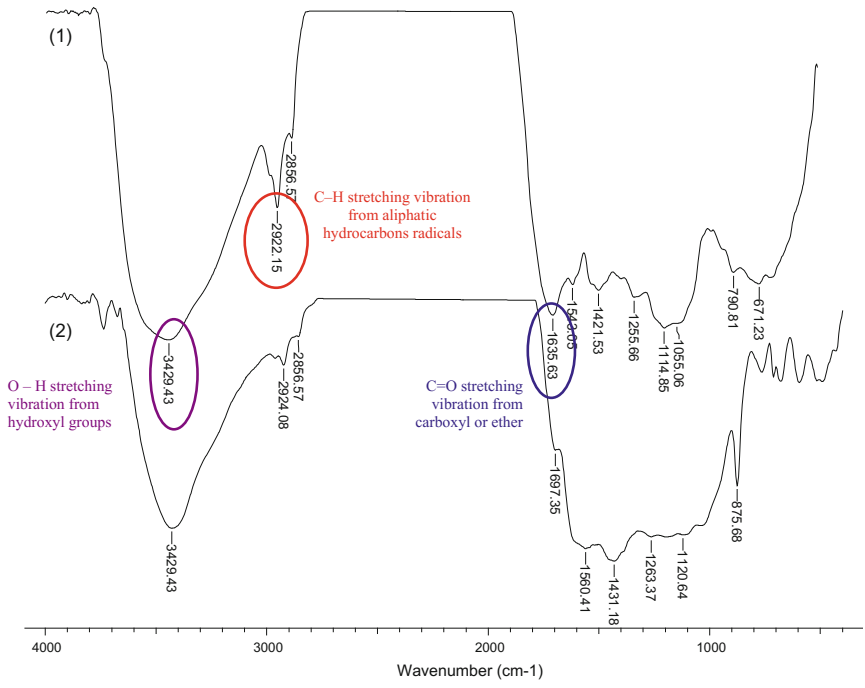

Fig. 6 IR spectra of *Ulva lactuca* sp. marine green algae biomass before (**1**) and after (**2**) thermal treatment

The analysis of IR spectra it allows us to say that

(i) in the structure of marine algae biochar are still sufficient functional groups that can bind heavy metal ions from aqueous media—so such material can be used as biosorbent for the removal of heavy metal ions from aqueous media;
(ii) due to the thermal treatment, the biochar has a high specific surface area, which may also represent an advantage in the heavy metals biosorption processes.

4.2 *Evaluation of Biosorptive Performances of Marine Algae Biochar*

In order to evaluate the biosorptive performances of marine algae biochar it was considered the influence of initial metal ions concentration and contact time on the biosorption efficiency. The experimental results presented in literature (Johansson et al. 2016; Park et al. 2016) have shown that in comparison with marine algae biomass, the marine algae biochar has higher biosorption capacity, in the same experimental conditions. In Fig. 7 is presented for exemplification the variation of biosorption capacity (q, mg/g) as a function of Pb(II) concentration in case of *Ulva lactuca* sp. marine algae biomass and biochar obtained from this biomass after thermal treatment at 450 °C during of 4 h.

It can be observed that the biosorption efficiency of marine algae biochar increases rapidly as a function of the initial Pb(II) concentration, and there was no evidence that the biosorbent attains the saturation, for entire initial Pb(II) concentration range.

The good biosorptive performances of marine algae biochar, obtained in this case, has probably mainly two causes: (i) the formation of very high number of mesopores and macropores in the structure of biochar, which are responsible by the

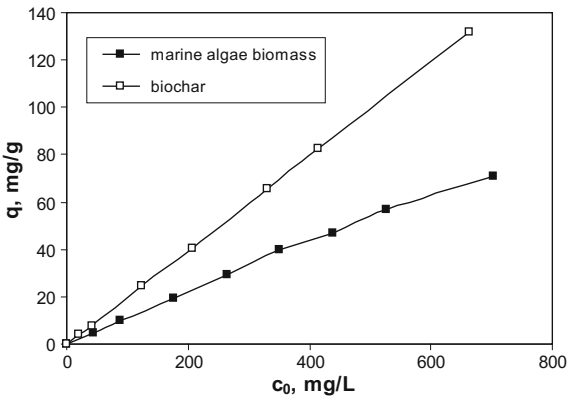

Fig. 7 Influence of initial metal ions on Pb(II) biosorption on *Ulva lactuca* sp. marine green algae biomass, before and after thermal treatment

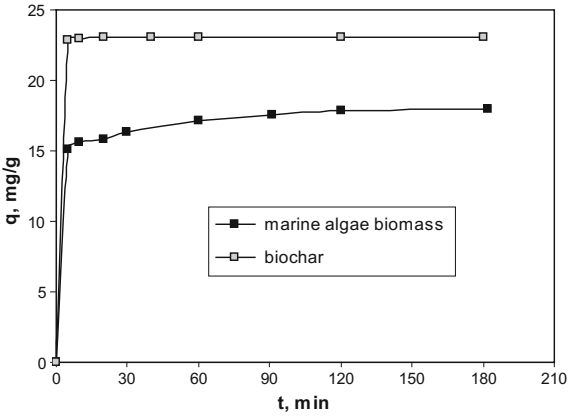

Fig. 8 Influence of contact time on Pb(II) biosorption on *Ulva lactuca* sp. marine green algae biomass, before and after thermal treatment

increase of specific area of this biosorbent, and (ii) the increase in the quantity of exchangeable cations by activation, which has an important contribution to the biosorption process of Pb(II) ions (Bulgariu 2016; Park et al. 2016).

The equilibrium modelling of experimental isotherms obtained by using marine algae biochar shows that and in this case the Langmuir model is the most adequate for the mathematical description of heavy metal ions biosorption, and the values of maximum biosorption capacity (q_{max}, mg/g) necessary for the formation of monolayer coverage are much higher than those obtained in case of utilization of marine algae biomass as biosorbent, in the same experimental conditions Thus, in case of Pb(II) biosorption on marine algae biochar the value of q_{max} was 5.32 times higher than the value obtained for using marine algae biomass as biosorbent (Bulgariu 2016).

Another important advantage of using marine algae biochar for the removal of heavy metal ions from aqueous media is the required time to attain the equilibrium of biosorption process is very short. For example, in case of Pb(II) ions the maximum removal efficiency (>95%) was obtained within 5 min in case of marine algae biochar, in comparison with 60 min, when marine algae biomass is used as biosorbent (Fig. 8).

Similar results have been also reported in literature for the biosorption of various heavy metal ions biosorption on such biochars (Jung et al. 2015; Inyang et al. 2016; Kim et al. 2016). In addition, in all these cases the kinetics data comply with the pseudo-second-order kinetics model, which suggest that the biosorption process has a certain selectivity degree, due to the chemical interaction which is the rate-controlling step.

In addition, the result reported in literature suggests the possibility of using marine algae biochar for the removal of heavy metal ions from accidental polluted waters. Thus, the low value of contact time required to attain biosorption equilibrium state indicate that immediately after adding the marine algae biochar the heavy metal ions are quantitatively retained on biosorbent surface. After the biosorption

process is ended, the biochar particles covered with heavy metal ions can be easy removed from waters by filtration, and thus the pollution risk of environment is eliminated.

4.3 Overview on Marine Algae Biochar Biosorbents

The use of marine algae biochar as biosorbent for the removal of heavy metal ions from aqueous media is a relatively new research direction, which has been more intensively developed in the last few years. From this reason, experimental studies must be still carried out in order to obtain a general picture over the biosorptive performances of this kind of biosorbent material in the environmental remediation processes.

Until now, the experimental results are encouraging, and highlight at least two advantages for the utilization of marine algae biochar as biosorbent for the removal of heavy metal ion, namely: (i) this material is a waste resulted from the production of green energy where as feedstock is used marine algae biomass, which is also a low-cost material, and (ii) the biosorption efficiency of the biochar obtained after thermal treatment of marine algae biomass seems to be higher in comparison with initial material.

5 Final Remarks

The removal of heavy metal ions from aqueous media by biosorption using marine algae biomass as biosorbents, has several important advantages over conventional wastewater treatment methods, including: high efficiency of heavy metal removal from dilute solutions; minimization of chemical and/or biological waste; economic viability; ease of use; etc.

Marine algae biomasses are usually considered low-cost materials and their utilization as biosorbents is determined by the large number and variety of functional groups from biomass surface, which is complemented by the relatively small and uniform distribution of binding sites on biosorbent surface.

Although the algae biomass have been proved to be an excellent biosorbent for heavy metals removal, this utilization is not very efficient from economic considerations. More efficient from this point of view it is the utilization of marine algae biomass as feedstock for energy production, and from this reason larger quantities are used for this purpose.

In literature are described at least two ways in which the marine algae biomass can be used for the obtaining of energy, namely: (i) extraction of oil from marine algae biomass and then its transformation in biofuels, and (ii) combustion of algae biomass through a gasification process at relatively low temperatures, the resulting

gases can be then used to obtain electricity. Both procedures have numerous advantages what have caused the intensification of researches in this field.

Unfortunately, even if the obtaining of energy from marine algae is considered a 'clean technology', the valorisation of algae waste resulted both after oil extraction and low temperature combustion is still important issue for which further solutions are sought. In this context, the utilization of such marine algae wastes as biosorbent for the removal of heavy metal ions from aqueous media besides that will ensure the utilization of such materials in agreement with the principles of sustainable development will be also helpful in the environment bioremediation processes.

Acknowledgement This study was elaborated with the support of a grant of the Roumanian National Authority for Scientific Research, CNCS—UEFISCDI, project number PN-III-P4-ID-PCE-2016-0500, Contract 41/2017.

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Selective Metal Ion Homeostasis in Cyanobacteria

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Abstract Metal homeostasis systems are responsible for the uptake and efflux of both essential and non-essential metals. The capacity of these systems to acquire a particular metal, whilst excluding another is essential for the survival of not just cyanobacteria, but all organisms. The initial step in the acquisition of metal ions from the environment is the physiological binding, or adsorption, of metals to cells. The second step, often energy expensive, is the internalisation of metals, which is facilitated by uptake systems. Metal release from cells requires an efflux system. Both uptake and efflux systems may be controlled by their own regulatory elements. The effectiveness of these transport systems is dependent upon their ability to discriminate effectively between metals. This discrimination is achieved largely by the proteins involved comprising of different metal coordinating ligands strategically positioned in the tertiary structures. For cyanobacteria, arguably the most adept organisms at survival on earth, the information on metal coordination and binding is still limited. However, studies identifying and providing functional characterisation of metal transporters and metalloproteins in cyanobacteria are contributing new insights into metal homeostasis across all living organisms.

Keywords Cyanobacteria · Trace element · Metal homeostasis · Metal transporters · Ligands

1 Introduction

For cyanobacteria, the trace elements cobalt, copper, iron, manganese, magnesium, nickel and zinc are essential for cellular metabolism. Such metals are only required at trace (ppb-ppm) levels, with either deficiency or over-abundance of these metal ions reducing cell viability and potentially leading to death. Non-essential metals

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B.N. Tripathi and D. Kumar (eds.), *Prospects and Challenges in Algal Biotechnology*, DOI 10.1007/978-981-10-1950-0_7

such as: arsenic, cadmium, chromium and lead are not required by cells, and whilst they may be tolerated at low concentrations, they have overall deleterious effects on cellular metabolic activity resulting in death. Metal levels in the environment fluctuate greatly. In soils, metal levels can range from less than 1 ppm (0.0001%), through to 100,000 ppm (10%) or higher in extreme examples, such as mineral ore deposits (Olajire et al. 2003; Wuana and Okieimen 2011; J. Halili et al. 2013; Nessa and Jewel 2014).

Cyanobacteria (Cyanophyta) are arguably the most diverse phylum exemplified by *Prochlorococcus marinus* and *Nostoc punctiforme*. *Prochlorococcus* spp. typically represents 50% of total chlorophyll in sub-tropical marine waters, contributes 30–80% of the total photosynthetic activity in oligotrophic oceans, physically are the smallest known phototrophs (at 0.5–0.7 μM) and have a genome size of only ~1.8 Mb (Partensky et al. 1999). In contrast, *N. punctiforme* is a filamentous multicellular species that produces vegetative cells, heterocysts, akinetes and motile hormogonia; inhabits fresh water and soil environments; grows in free-living or in symbiosis with a wide range of hosts; has cells ranging in size from 2 to 10 μM (depending on water activity in its environment and cell type) and has one of the largest prokaryote genomes at ~10 Mb including plasmids (Meeks et al. 2001; Villarreal and Renzaglia 2006).

Over 3.5 billion years of evolution has revealed that cyanobacteria are adept at survival, and can thrive in most environments. As the earth and its environment evolve, cyanobacteria also continue to evolve as they did when they facilitated the earth's transformation into the oxygenated atmosphere that enabled oxygen-dependent organisms to evolve. As our climate constantly alters, cyanobacterial stress response mechanisms provide invaluable insights into the biochemical changes that are occurring and the cellular processes that facilitate their survival and prosperity. Environmental shifts in metal abundance and distribution will occur as more metal contaminants are introduced and spread through anthropogenic activities and as water bodies' undergo more extreme drying and flooding events. As our climate alters, metal levels will become more highly concentrated as water levels decrease, due to increased drought. This will have adverse effects on many biota. Understanding the cellular metal uptake and efflux pathways in cyanobacteria provides invaluable insights into biological responses to climate changes.

Using cyanobacteria, potential models representing the cellular mechanisms for maintenance of homeostatic metal levels can be developed and manipulated, with applications to the development of cyanobacteria as bioremediation or bioleaching tools. Moreover, understanding the system involved in maintaining homeostatic metal levels provides further insights as to the response mechanisms in cyanobacteria, and for the case of cyanobionts such as *N. punctiforme*, may provide insights into strategies to increase metal availability or metal resistance to a host plant during symbiosis. Whilst it has been previously identified that symbiotic cyanobacteria may increase metal availability to host plants, a general consensus among current literature supports that cyanobacteria are more likely to increase plant resistance to high levels of metals, rather than increasing metal availability

(Babich and Stotzky 1978; Brown and Beckett 1983; Dupont et al. 2010). Symbiotic *N. punctiforme* in the coralloid roots of cycads may potentially increase the capacity of plants to tolerate and grow in otherwise growth-disrupting metal levels (Nilsson et al. 2006).

2 Cellular Metal Ion Requirements

Trace elements including cobalt, copper, manganese, nickel and zinc are essential for cellular processes including enzyme catalysis, protein folding and signalling (Baptista and Vasconcelos 2006; Ma et al. 2009). The maintenance of homeostatic levels of trace elements is crucial for normal cellular functioning and growth (Tripathi et al. 2003; Yamamoto and Ishihama 2005). The trace element requirements for cyanobacterial cells vary greatly in contrast to most other prokaryotes; having a much higher quota required for optimum growth (Shcolnick and Keren 2006). This is largely attributed to the metal quota required for optimal functioning of the two large multi-subunit pigment protein complexes—photosystems I and II (PSI and PSII) (Zak et al. 2001). For cells actively growing under diazotrophic (fixing atmospheric nitrogen) conditions, insufficient availability of the trace elements iron and molybdenum limits growth (Howarth et al. 1988).

The acquisition of trace elements by cyanobacteria can be separated into two distinct processes: First, the passive adsorption of metals to cells and second, the active internalisation by transporters. The production of chelates, such as siderophores, enables cells to scavenge for trace elements under "low" or "stressed" conditions (Neilands 1995). Siderophores predominantly solubilise ferric iron (Fe^{3+}), but to a limited extent also ferrous iron (Fe^{2+}) and other divalent cations. They are small molecules, secreted by cells to chelate the iron or metals and interact with select membrane transport channels to import trace elements (Neilands 1995).

Whilst it is essential for cells to have an adequate supply of trace elements to ensure optimal growth, intracellular metal levels must be regulated to avoid toxic levels of accumulation. The maintenance of homeostatic levels of metals is achieved by regulation and suppression of the active uptake pathways, and through efflux systems.

3 Metal Ion Adsorption

The adsorption of metals to cells is the initial step in the acquisition of metals from the environment. Investigation of the physiological metal uptake by *N. punctiforme* has demonstrated that the majority of zinc, cobalt, copper, manganese, nickel and cadmium associated with cells were initially adsorbed (Hudek et al. 2012). This has also been demonstrated in other physiological metal uptake studies for

cyanobacteria, where the initial adsorption of free metal ions to the cyanobacterial cell surfaces occurs rapidly, and is largely based on charge (as cells have an overall negative net charge) (Dickson and Koohmaraie 1989). Beyond the initial adsorption of metal to cells, the internalisation of metal intracellularly is much slower, energy dependent and more selective for different metals. In most bacteria, including cyanobacteria, total trace metal levels per cell are within the millimolar scale (10^{-3}), whilst the intracellular levels that elicit changes in the expression of regulatory elements for active uptake and efflux channels are in the femtomolar scale (10^{-15}) (Outten and O'Halloran 2001; Outten et al. 2001; Kranzler et al. 2014; Sharon et al. 2014). For an individual metal such as zinc, iron or manganese, the intracellular levels of free metal have been shown to be sub-attomolar (10^{-18}) (Outten and O'Halloran 2001; Outten et al. 2001; Kranzler et al. 2014; Sharon et al. 2014). Maintaining homeostatic levels of intracellular trace metals requires the balancing of uptake and efflux transporting proteins expression. When elevated levels of free intracellular zinc, manganese or another metal become too high, to avoid the toxic over-accumulation, cells use regulated efflux transporters embedded in the cytoplasmic membrane (Ma et al. 2009).

4 Molecular Basis of Metal Ion Homeostasis

Metal homeostasis systems are responsible for the uptake and efflux of both essential and non-essential metals. The ability of these systems to acquire a particular metal, whilst excluding another is essential for the survival of not just cyanobacteria, but all organisms. Intracellular metal homeostasis in prokaryotes, including cyanobacteria, is based on uptake and export systems, which are controlled by specific regulators (Tripathi et al. 2003; Yamamoto and Ishihama 2005).

The Adenosine Triphosphate (ATPase) Binding Cassette (ABC type) metal uptake systems, have been established to be vital for metal uptake in bacteria (Fath and Kolter 1993; Patzer and Hantke 1998; Blencowe and Morby 2003; Linton and Higgins 2007). This family of metal uptake transporters includes the Adc, Czc, Mnt, Psa, Sit Tro, Zit and Znu of *Alcaligenes eutrophus*, *Escherichia coli*, *Lactococcus lactis*, *N. punctiforme*, *Streptococcus pneumoniae*, *Sinorhizobium meliloti*, *Synechocystis* sp. and *Treponema pallidum* (Nies 1992; Dintilhac et al. 1997; Patzer and Hantke 2000; Platero et al. 2004; Ammendola et al. 2007; Desrosiers et al. 2007; Nies 2012; Hudek et al. 2013b). The specificity for metal transport by these systems is broad, with the affinity and preferred allocrite transported not fully understood. Of these systems, the Znu is potentially the best understood for cyanobacteria (Fig. 1a, b).

Based on the structure of the metal-binding site, that consists of conserved histidine residues, the Znu zinc uptake system is classified as belonging to the cluster 9 family of metal transporters (Banerjee et al. 2003). The ZnuB component of the ZnuABC system is the cytosolic membrane-spanning component of the

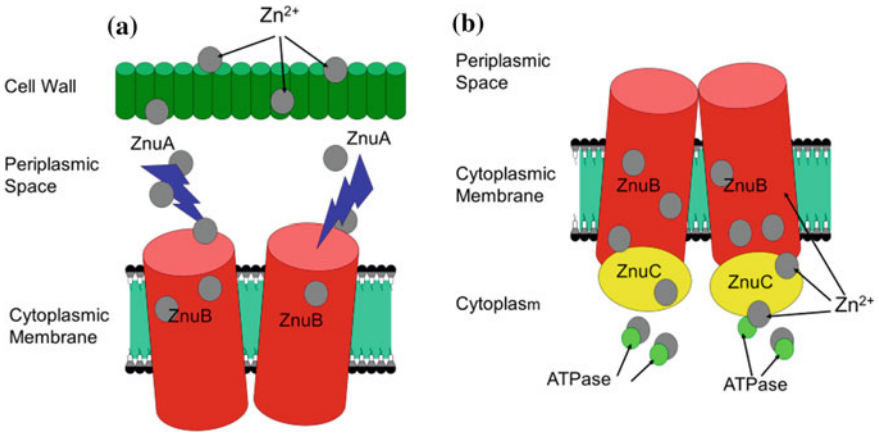

Fig. 1 One type of zinc uptake system in cyanobacteria is the ATP-binding cassette (ABC)-type zinc uptake complex, which comprises three genes: ZnuA, B and C. The Znu system is transcriptionally repressed by the broad substrate-responsive ferric uptake regulator (Fur), and the zinc uptake regulator (Zur) (modified from Cerasi et al. (2013)) (Cerasi et al. 2013). **a** ZnuA (shown in blue) is a soluble protein located between the cell wall and the periplasm that is the initial binding site for zinc. ZnuB (shown in red) is a membrane-bound protein, typically having eight transmembrane domains, which interacts with ZnuA to bring zinc from the periplasmic space, through the membrane. **b** ZnuC (shown in yellow) is a soluble protein located in the cytoplasm that interacts with both ZnuB and cytosolic proteins (shown in green). ZnuC binds zinc in the cytosol, thus making it metabolically active

system. ZnuB facilitates cation transport through the plasma membrane and its peptide sequence and structure can be indicative of the ligand specificity of the overall system (Linton and Higgins 2007). ZnuA and ZnuC are the two hydrophilic nucleotide-binding-domains (NBDs). These NBDs power the transport cycle through the well-described ATP-switch model (Dintilhac et al. 1997; Linton and Higgins 2007). The NBD switch operates via a dimeric confirmation, closed around two ATP molecules and a nucleotide-free dimeric open confirmation (Linton and Higgins 2007) (Fig. 1a, b).

Transcriptional regulatory mechanisms control the expression of ABC transporter genes, including the *znuACB* system. Transcriptional regulators that alter the expression of *znu* genes include the ferric uptake regulator (Fur), zinc uptake regulator (Zur) and the ArsR-SmtB group of regulons. These regulons are responsive to a number of divalent cations including Cd^{2+}, Co^{2+} and Zn^{2+} (Patzer and Hantke 2000). With regards to the *znu* system, the zinc sensing regulator Zur is predicted to be the dominant transcriptional repressor that interacts with this system (Patzer and Hantke 2000). Based on structural and functional similarities, Zur has been determined to be a member within the fur family of transcriptional repressors (Patzer and Hantke 2000).

Zur is a cytoplasmic protein that is widespread among bacteria and cyanobacteria (Patzer and Hantke 1998). Whilst Zur can control the activity of several high

affinity uptake systems, predominantly it regulates the *znu* system (Patzer and Hantke 1998). Previous functional investigations of Zur in *Streptomyces coelicolor*, demonstrated it negatively as regulated *znuA* gene expression, and interestingly was also shown to regulate the expression of itself (Owen et al. 2007).

Studies of the bacterial Znu system and its transcriptional repressor Zur have identified the functional domains within the protein tertiary structures including the metal-binding domains (Patzer and Hantke 1998, 2000; Lindsay and Foster 2001; Outten et al. 2001; Shin et al. 2007). This knowledge has enabled the modelling and understanding of metal transport, particularly for zinc, in cyanobacteria. In *N. punctiforme* the *znu* system is comprised of at least five genes, *znuA08*, *znuA18*, *znuB*, *znuC* and *zur*; with all these components having a role in transporting metals intracellularly. Whilst the *znu* system in *N. punctiforme* has been shown to be responsive to zinc, the expression of *znu* components also changed in cells exposed to cadmium, cobalt, copper, manganese and nickel. This finding provides insights as to this system having a broad allocrite range. This is consistent with previous investigations of other bacterial *znu* and *znu*-like ABC transporters. Overall, investigations of the Znu transporters in cyanobacteria are limited, in particular for *Synechocystis* and *Synechoccocus* species. Studies into the functional similarities between cyanobacterial metal transporters with other bacterial Znu transporters, such as those of *Escherichia coli*, *Salmonella*, *Streptococcus* and *Staphylococcus* species are limited (Phung et al. 1994; Patzer and Hantke 1998; Banerjee et al. 2003; Blencowe and Morby 2003; Liu et al. 2004; Chandra et al. 2007; Linton and Higgins 2007). Complementation studies have previously been performed using *E. coli znu*$^{-}$ and *zur*$^{-}$ mutants complemented with recombinant *N. punctiforme znuA08, znuA18, znuB* and *znuC* (Hudek et al. 2013b). The outcomes from this work indicated that to a limited extent the *N. punctiforme* ZnuA08, ZnuA18, ZnuB and ZnuC could restore zinc uptake in the corresponding *E. coli* mutants (Hudek et al. 2013b). Similarly, complementation of an *E. coli zur-* mutant strain with *N. punctiforme* Zur, reduced zinc accumulated by the complemented mutants, indicating that the Zur of *N. punctiforme* was capable of reducing the expression of zinc uptake systems such as *znu* in *E. coli* (Hudek et al. 2013b). Interestingly, the recombinant *N. punctiforme* Zur also reduced zinc accumulated by *E. coli fur-* mutants (Hudek et al. 2013b).

The solute carrier (*slc*) family is another uptake system responsible for the internalisation of metals in cyanobacteria. The *slc39* family, previously known as the *zip* (ZRT-, IRT-like Protein) family of genes and proteins, are capable of importing zinc in both prokaryotes and eukaryotes (Eide 2006). The Zip's are distinguished from the Znu transporters based on the isolation of *zip* genes from distinct operons and their functional role to transport high amounts of zinc under low physiological conditions; unlike the *znuACB*, which exist in an operon and transport zinc at low levels under optimal conditions (Barnett et al. 2012; Napolitano et al. 2012; Hudek et al. 2013b). Members of the Slc39 (Zip) family of metal uptake proteins are distinguished by the presence of eight transmembrane domains with conserved positioning (Eide 2006). Typically the N-and C-termini of

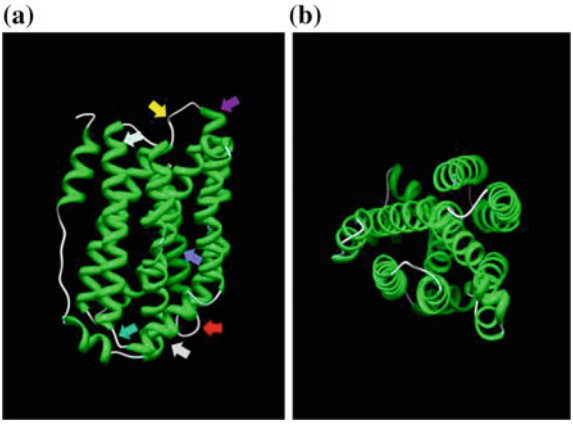

Fig. 2 Structural prediction of a Zip from *N. punctiforme* showing amino acid residues predicted to be a part of zinc-coordinating and binding regions and predicted α-helices (green) and loops (white) modified from Hudek et al. (2013a). **a** Front view showing His^{93} (blue arrow), His^{196} (yellow arrow), His^{230} (red arrow), Glu^{99} (grey arrow), Glu^{154} (orange arrow), Glu^{200} (purple arrow) and Asp^{67} (aqua arrow). **b** Top view

Zip proteins positioned on the extracellular side of the membrane (Eide 2006). A conserved characteristic of Zip proteins is a long loop region located between the third and fourth transmembrane domains. This loop commonly contains a histidine-rich sequence, which is suggested as being the binding site for zinc and other metals (Eide 2004, 2006). Based on the established characteristics for Zip transporters, the protein structures of the Zip transporters in *N. punctiforme* have been estimated along with the metal-binding amino acids (Hudek et al. 2009) (Fig. 2a, b).

Only recently have the metal efflux systems been investigated in cyanobacteria, and little is known about their functional similarities to the other characterised bacterial Slc39 homologues such as ZupT of *E. coli*. ZupT, was the first bacterial Slc39 transporter to be identified and characterised (Taudte and Grass 2010). Most bacterial and cyanobacterial metal transport systems have been shown to have a capacity to transport more than one metal. The specificity for the metal transported is dependent on the orientation of specific amino acid resides (such as cysteine for copper or histidine for zinc) within peptide sequences and tertiary structures (Ma et al. 2009). Previously the substitution of zinc-coordinating histidine residues to alanine and tyrosine the zinc-binding Staphylococcal enterotoxin C2 (SEC2) was shown to inhibit zinc-binding to C2, confirming the functional role of zinc-binding by histidines residues (Papageorgiou et al. 1995; Wang et al. 2009). The ZupT of *E. coli* is broad known to transport multiple metals; including cadmium, cobalt, iron, manganese and zinc (Taudte and Grass 2010). Previous studies have shown that cadmium inhibits the transport of zinc by ZupT, suggesting that ZupT preferentially transports cadmium over zinc (Ma et al. 2009; Taudte and Grass 2010).

Cadmium uptake resulting in cellular stress from high intracellular levels may be further exacerbated under zinc deficient conditions, due to the zinc being out-competed by cadmium (Ma et al. 2009; Taudte and Grass 2010).

Highly efficient metal efflux systems are the saving measure for reducing the potentially toxic over-accumulation of in cells. These unique transport systems may complement the uptake systems, enabling the maintenance of homeostatic levels of metals by cells. These active efflux channels are embedded in the cytoplasmic membrane, and through regulation evacuate metals out of the cells—thus reducing intracellular metal levels. One family of transporters well established to actively pump metal ions, including zinc, from the cytoplasm in both eukaryotes and prokaryotes is the Cation Diffusion Facilitator (CDF) family (Palmiter and Huang 2004; Eide 2006). Typically, Cdf proteins are characterised by the presence of six transmembrane domains (Eide 2006). In bacteria the CDF family includes the zinc transporting proteins YiiP, Znt, Zit and the cobalt-zinc-cadmium transporting Czc (Anton et al. 2004; Neis 2007). The CzcD protein of *Ralstonia metallidurans* was among the first bacterial CDF systems characterised (Anton et al. 2004; Neis 2007). The CzC of *R. metallidurans* and the ZitB of *E. coli* have both been shown to have functional roles in metal efflux and resistance for a number of metals including zinc, cobalt, cadmium and nickel, but not magnesium or manganese (Anton et al. 2004). In *E. coli*, the YiiP has been described as being able to compensate for the Czc proteins inability to transport magnesium, with it being demonstrated to have an affinity for both magnesium and zinc (Neis 2007). Whilst the ZntA from *E. coli* is established to be a zinc efflux protein, it has also shown to transport a number of metals including cadmium, cobalt, copper, lead and nickel (Hou and Mitra 2003). The broad range of metal ions transported by CDF members in other bacterial systems including *E. coli* and *R. metallidurans* has also been demonstrated for *N. punctiforme* Cdf's (Hou and Mitra 2003; Anton et al. 2004; Neis 2007; Hudek et al. 2015b).

The characterisation of cyanobacterial CDFs in *N. punctiforme*, revealed the presence of four distinct *cdf* genes, two of which evacuate zinc from cells (Hudek et al. 2015b). The presence of the multiple *cdf's* is an example of the redundancy of genes in *N. punctiforme* where they can complement each other and ensure safe guarding of homeostatic intracellular metal levels (Hudek et al. 2015b).

The NpunR4017 (ZntA) is a zinc efflux transporter in *N. punctiforme* is crucial for maintaining normal intracellular levels of zinc. The NpunR4017 transporter was shown to be transcriptionally altered in cells exposed to elevated zinc levels based on both microarray and qRT-PCR experiments. Using radiolabeled ^{65}Zn, the function of NpunR4017 was shown to mediate zinc efflux in *N. punctiforme*, and complement the function of the ZntA in *E. coli* to a limited extent (Hudek et al. 2015a). The deletion and over-expression of NpunR4017 altered gene expression of *zip11, zip63* and the four CDF transporters in *N. punctiforme*, reiterating its redundancy in duplicated genes provide assurance of homeostatic intracellular metal levels are maintained (Hudek et al. 2015a).

5 Coordination of Metal Binding by Proteins

The binding of divalent cations by proteins, in particular metalloproteins, is based on the order of affinity as defined by the Irving-Williams series ($Mg^{2+} < Ca^{2+} < Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$) (Waldron and Robinson 2009; Huertas et al. 2014). This binding order presents a great challenge for the binding of a specific metal by a certain protein (Waldron and Robinson 2009; Huertas et al. 2014). Metal-binding sites in proteins vary in their coordination geometry and entity (Ibers and Holm 1980; Yamashita et al. 1990). This includes their backbone carbonyl oxygens; sidechain groups of aspartic, asparagine, cysteine, glutamic, glutamine, histidine, methionine, serine, threonine, and tyrosine residues; and water molecules (Ibers and Holm 1980; Yamashita et al. 1990). This is exemplified by ferrous iron (Fe^{2+}) bonding to sulphur ligands from the side chains of cysteine and methionine residues (Yamashita et al. 1990; Nemirovskiy and Gross 1998). The binding of iron is not restricted to cysteine and methionine, and the presence of alternate amino acids can change the binding specificity to include other metals in addition to iron. In eukaryotes, the mitochondrially produced protein frataxin is established as being able to bind intracellular iron (Pastore et al. 2007). Aside from binding iron, frataxin has low cation specificity and contains multiple binding sites that can chelate both divalent and trivalent metals, such as ferric iron (Fe^{3+}) or aluminium (Al^{3+}), with low affinity (Pastore et al. 2007). The diversity in metals bound by frataxin is attributed to its presence of exposed glutamates and aspartates, which are residues that are unusual for iron chelation when not accompanied by histidines or cysteines (Pastore et al. 2007). The same principles described for Frataxin apply to siderophores; where the ligand coordination alters the specificity and capacity to chelate either ferric or ferrous iron, or other divalent cations (Neilands 1995).

In addition to the binding of metals by specific amino acids, amino acids located within the tertiary structures such as cavities or pockets can coordinate and bind metals, and alter the protein's metal-binding properties. Differences in coordinating ligands and protein environments change the redox potential of iron impinging on its binding potential. This results in other metals being able to outcompete the iron at the binding site, thus providing a protein with broad specificity (Shouldice et al. 2004, 2005; Pastore et al. 2007).

For the Znu, Zip, Cdf and Znt transporters, the coordinating ligands and their positioning is crucial for the preferential binding of one metal over another (i.e. zinc > manganese) (Fig. 2a, b and 3a, b) (Eide 2006; Ma et al. 2009; Hudek et al. 2013a; Jeong and Eide 2013; Hudek et al. 2015b). The positioning of histidines, cysteines, glutamates and aspartates with regards to the cell membrane (inside or outside) are a key identifier for transporters and provide initial ways to classify transporters into a family (Eide 2006; Ma et al. 2009; Jeong and Eide 2013). For cyanobacteria, there are no structurally resolved metal transporters, with the only examples being in silico-determined predicted models (Blindauer 2008; Ma et al. 2009; Hudek et al. 2013a, b, 2015b; Barnett et al. 2014) (Figs. 2a, b and 3a, b).

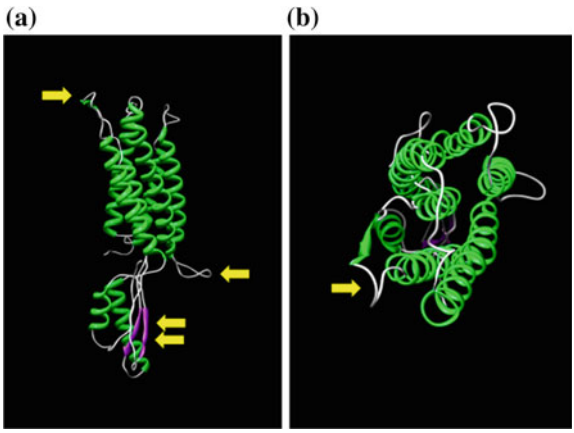

Fig. 3 Structural prediction of a Cdf from *N. punctiforme* showing histidine residues predicted to be a part of zinc-coordinating and binding regions (yellow arrows). Also predicted are the α-helices (green), β-sheets (purple) and loops (white) (modified from Hudek et al. (2015b). **a** Front view **b** Top view

The use of site mutagenesis for substitution of amino acid residues within metal-binding and transporting proteins is the most common and informative for determining metal-binding ligands; largely due to the complexity, instrument limitations and/or low success of structural resolution by either nuclear magnetic resonance (NMR) or x-ray crystallography based structural determination methods. Characterisation of the highly conserved Met, His and Tyr residues involved in copper binding by the copper importing CcoA of *Rhodobacter capsulatus* was achieved using site mutagenesis. Based on the binding of radiolabeled copper by CcoA mutants, the order of importance of individual amino acids on copper binding was determined to be: Met^{233} and His^{261} essential, Met^{237} and Met^{265} important, and Tyr^{230} no role (Khalfaoui-Hassani et al. 2016). The number of studies identifying and providing functional characterisations of metal transporters and metalloproteins from cyanobacteria are increasing. The next step beyond this is the determination of the metal coordinating and binding ligands as well as determination of tertiary structures.

6 Conclusion

In the face of a rapidly changing climate, understanding of the molecular basis of metal ion homeostasis in cyanobacteria will provide insights into how one of the most adept organisms has survival on earth, having overcome over 3.5 billion years of environmental change. Elucidation of the complex metal ion homeostatic mechanisms present in cyanobacteria will contribute to an understanding of metal transport in many other organisms as the transport systems are highly conserved across different life forms.

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Bioadsorption of Heavy Metals

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Abstract Pollution caused by heavy metals is one of the most serious environmental problems for society. Industrial activities increase the concentration of heavy metals such as Cu(II), Cd(II), Zn(II), Pb(II) and Ni(II) in aquatic systems and mainly in the fields of mechanics, electrics, electronics, tanning, galvanization, oil industries and mining. Biomagnification of these metals occurs through the toxicity of the trophic for humans. As a remedial measure, it is for scientists to find new biosorbents which are able to ameliorate the possible toxic effects of heavy metals in water bodies. Studies of bioadsorption have identified this as a real alternative to wastewater treatment, especially for the removal of heavy metals. This chapter explores (1) the characterization of new biosorbents via surface acid–base titration, where the type of functional groups can be tentatively computed, (2) kinetics of bioadsorption (pseudo-first and second order), (3) bioadsorption as a function of pH and (4) bioadsorption as a function of metal concentration in solution (Langmuir, Freundlich, Sips, Redlich–Peterson, Tóth, Frumkin and Temkin isotherms), where the maximum adsorption capacity can be determined under different experimental conditions. The majority of bioadsorption studies have been carried out at laboratory scale; however, future studies will be conducted at industrial scale as a way to

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B.N. Tripathi and D. Kumar (eds.), *Prospects and Challenges in Algal Biotechnology*, DOI 10.1007/978-981-10-1950-0_8

remediate heavy metal pollution in water bodies. Different commercial biosorbents and their characteristics are presented in this chapter.

1 Introduction

Environmental pollution is one of the most pressing issues for our society, and one of the unsolved questions in this respect is how to decrease pollution by heavy metals, especially in water bodies where these metals are released as a result of the increased industrial activity. The presence of heavy metals such as Cu(II), Cd(II), Zn(II), Pb(II) and Ni(II) is directly linked to their use in a number of industrial activities such as mechanics, electrics, electronics, tanning, galvanization, oil industries or mining (Abdel-Ghani and El-Chaghaby 2014), being toxic for humans and ecosystems (Meena et al. 2008). These metals are not biodegradable and have long resident times in aqueous environments. This environmental phenomenon is of particular significance for humans due to the biomagnification of these metals through the trophic web, creating gastrointestinal, neurological and immunological hazards. Therefore, it is vital for the scientific community to continue research into new biosorbents in order to ameliorate the effect of heavy metal pollution in water bodies, aiming at identifying economical and eco-friendly biosorbents.

Since the 1950s, great advances have been made in the study of sorption of heavy metals on different biomaterials, aiming at finding an efficient, effective, economical and eco-friendly biosorbent. During this time, the knowledge about complex biosorption mechanisms and models has been enhanced. In terms of patenting, bioremediation has achieved 237 patents since 1975 (Jara et al. 2016). 137 of these patents and more than 1500 research papers have addressed algae as biosorbents due to their high adsorption capacity (Michalak et al. 2013). However, most of this scientific work has taken place at laboratory scale, making it necessary to transfer it to the industrial scale. Bioadsorption is becoming a potential alternative for wastewater treatment, especially for the removal of heavy metal, even though a lot remains to be learned before application to the industrial scale can be considered, especially due to the low stability and low mechanical resistance of the biomass. Bioadsorption holds many advantages compared to other types of treatment, for instance, high efficiency for low metal concentrations, efficiency for a wide range of pH and temperature conditions, easy recuperation of adsorbed material, as well as easy and cheap production of biomass. Bioadsorption offers the added advantage of working with non-living biomass, reducing the possibility of contamination, toxic effects and metabolic activities.

It is important to distinguish between bioadsorption and bioaccumulation since both are commonly used to remove heavy metals from solutions:

Bioadsorption is a physico-chemical process in which the concentration of sorbate (heavy metals) is adsorbed on the bio-surface. In general, this biological surface is not living biomass. Consequently, it is a metabolically passive

mechanism, a rapid and reversible mechanism binding metal ions from aqueous solutions onto functional groups.

Bioaccumulation or bioabsorption refers to a sorbate accumulated into the cells. Therefore, bioaccumulation is a metabolically active process which follows the first step, bioadsorption.

The adsorption of heavy metals on the surface of a biosorbent is a physico-chemical mechanism including different processes such as electrostatic interaction, complexation, ion exchange or proton displacement (Davis et al. 2003; Volesky 1990; Crist et al. 1999). This adsorption process is affected by many physico-chemical conditions, not only those affecting the biosorbent but also those of the sorbate and media such as molecular weight, ionic radius, oxidation state, the concentration of both adsorbent and sorbate, biosorbent properties, pH, temperature, ionic strength, etc.

In order to propose a new biosorbent, it should be precisely characterized in terms of adsorption. This requires consideration of the presence of functional groups on the cell surfaces, adsorption capacities, both as a function of pH and metal concentration in solution, kinetics of adsorption, as well as other factors such as temperature and biomass.

2 Characterization of Biosorbents

2.1 Surface Acid–Base Titration

Surface acid–base titrations provide a measure of sequential binding of protons by the various functional groups on the surface of the algae, and the variation in that portion of the charge caused by H^+ binding (González-Dávila 1995; González-Dávila et al. 1995; Stumm and Morgan 1981). This allows us to quantify the proton and hydroxyl buffer capacities of biosorbents in a wide range of pH and thus determine the proton binding capacity and the concentration of amphoteric surface functional groups on biosorbents.

The excess surface proton concentration can be calculated as (Martinez et al. 2002; Cox et al. 1999; González et al. 2010; González and Pokrovsky 2014):

$$[H^+]_s = \left(\left(C_{aj(suspension)}\right) - \left(C_{aj(reference)}\right)\right) - \left(\left(C_{bj(suspension)}\right) - \left(C_{bj(reference)}\right)\right) \quad (1)$$

where C_{aj} and C_{bj} correspond to the concentration of base and acid for the jth addition of titrant, respectively. As a reference system, the supernatant from the rinsed biosorbent biomass is used and processed exactly in the same way as biosorbent suspension. Note that this definition of excess surface proton concentration does not allow direct conversion to the cell surface charge, as the initial amount of surface protons is not known.

The acid–base titration experiments have been modelled by considering that the proton dissociation mechanisms for a single protonated site correspond with the following:

$$HL \rightarrow^{K_a} H^+ + L^- \tag{2}$$

where HL are the protonated binding sites on the surface and H^+ is the hydrogen concentration measured directly with a calibrated pH electrode. L^- is the deprotonated surface reactive site with a net negative charge. Consequently, the apparent proton dissociation constant can be expressed as:

$$K_a = \frac{[L^-][H^+]}{[HL]} \tag{3}$$

pK_a is defined as $-\log K_a$.

The Linear Programming Model (LPM) created by Martinez et al. (2002, 2004) and Martinez and Ferris (2001) can be used to rationalize experimental results in order to obtain the main parameters of surface equilibrium. This method also takes into account the intrinsic affinity constant. Each reactive group in the cell wall of the biosorbent would have a characteristic intrinsic affinity constant (Martinez et al. 2002). This method allows minimizing the number of binding sites and the absolute error, rather than the least squats. However, LPM is a mathematical solution without any base in chemistry. In the case of surface acid–base titrations, a matrix is constructed by considering the experimental net surface charge excess ($b_{meas,i}$) for the ith addition of titrant expressed as:

$$b_{meas,i} = C_{bi} - C_{ai} + [H^+]_i - [OH^-]_i \tag{4}$$

C_{ai} and C_{bi} are the acid–base concentrations at the i*th* addition of titrant. Finally, the calculated net surface charge excess can be written as follows:

$$b_{calc,i} = \sum_{j=1}^{m} \left(\frac{[L_T]_j K_{a,j}}{K_{a,j} + [H^+]_i} + S_0 \right) \tag{5}$$

Here, m corresponds to the number of binding sites and $[L_T]$ with the site density. $K_{a,j}$ is the apparent acidity constant for the jth site, and S_0 is a constant which is necessary in order to account for positive changes on the surface (Brassard et al. 1990; Smith and Ferris 2001).

Cation-exchange capacity determined by acid–base titration is correlated with bioadsorption capacity evaluated via adsorption experiments. For example, if pH is higher than pK_a, functional groups suffer deprotonation and become available for metal ion interactions (Ofomaja and Ho 2007). The concentration of functional groups computed via titration is responsible for the heavy metal adsorption on the cell surfaces. Generally these functional groups on the cell wall, tentatively ranked according with the pK_a value, are carboxyl, sulfonate, phosphoryl, amino, polyphenols amide, imidazole (González-Dávila 1995; González-Dávila et al. 1995; González et al. 2010; González and Pokrovsky 2014; Volesky et al. 1999; Pokrovsky et al. 2008a). These groups are ranked according to with the pK_a value

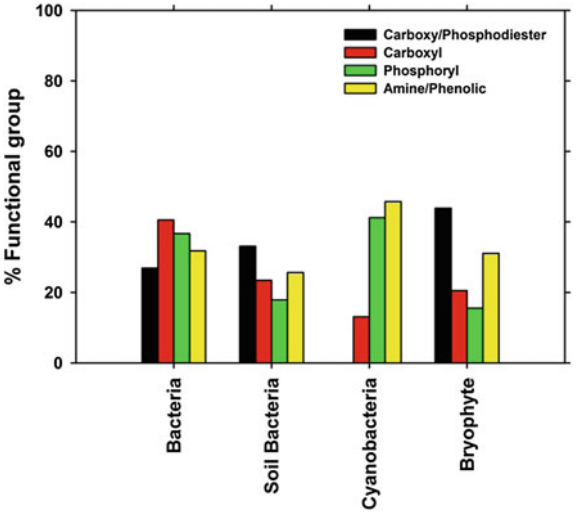

Fig. 1 Percentage of functional groups calculated via acid–base titration for different biosorbents as bacteria consortia (Borrok and Fein 2004), soil bacteria (González et al. 2010; Ueshima et al. 2008), cyanobacteria (Pokrovsky et al. 2008a; Martinez et al. 2008) and bryophytes (González and Pokrovsky 2014; González et al. 2016c)

computed from the acid–base titration. Figure 1 shows the concentration of functional groups for different biosorbents.

The composition of cell walls is very similar for most of the biosorbents, but small differences (in %) can have important effects in terms of bioadsorption. Biosorbents with high levels of carboxyl and hydroxyl groups are good sorbents, among them brown and green algae (Davis et al. 2003; Vieira and Volesky 2000), bacteria (Yee and Fein 2001) and bryophytes (González and Pokrovsky 2014). Gram-negative bacteria contain peptidoglycan and gram positive also teichoic acids (phosphoryl and hydroxyl groups). On the other hand, lower amounts of these groups and high levels of carrageen and sulfonate groups make them a less interesting biosorbent, for instance, red algae (Vieira and Volesky 2000).

Acid–base titrations also give us the pH values of the zero net proton adsorption (pH_{PZC}). This pH_{PZC} is directly linked to the chemical composition of the cell wall, and its variations between species can be understood in terms of its concentration of functional groups (Stumm and Morgan 1981; González-Dávila 1995; González-Dávila et al. 1995).

The chemical composition of the cell wall can also be studied with other techniques: IR, Raman, electron dispersive spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), electron microscopy (Scanning, Transmission), nuclear magnetic resonance (NMR), X-ray diffraction analysis and X-ray absorption spectroscopy (XAS) (Tsezos et al. 1997; Chojnacka 2010).

Particularly, the most interesting technique is XAS, including X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure region (EXAFS). It is the most powerful microscopic technique for the quantitative construction of the molecular environment, both organically and inorganically, of heavy metals bioadsorbed on the cell surfaces (Pokrovsky et al. 2012; González et al. 2016a).

2.2 Kinetics of Bioadsorption

The kinetics of bioadsorption gives us useful information for understanding the adsorption rate of heavy metal onto the cell surfaces and the necessary time for a full absorption process (Santana-Casiano et al. 1995). In fact, kinetic studies are necessary to select ideal conditions for adsorption experiments (Tuzen and Sarı 2010). In addition, this kind of experiments allows us to understand the reaction pathways and mechanisms of adsorption (Gupta and Bhattacharyya 2011). Bioadsorption is a quick process and normally the equilibrium is reached in the first few minutes (González-Dávila et al. 1995; González and Pokrovsky 2014). This means that the kinetic experiments should be carried out carefully in order to be able to fit an adequate kinetic model. The reaction order is related to the mechanisms of bioadsorption, mainly ion exchange or surface precipitation (metal hydroxide, sulphide or carbonate).

Different kinetic models have been applied to biosorbents, including pseudo-first and second order, considering non-equilibrium conditions (Xu et al. 2004; Ho et al. 2000; Erdem and Ozverdi 2006; Kiran et al. 2006; Rubín et al. 2006; Won et al. 2006; Kumar et al. 2005).

2.2.1 Pseudo-first order kinetic model

Also known as Lagergren model, it assumes that metal ions are bound only to one binding site on the cell surface (Ghaedi et al. 2013; Chojnacka 2010). The kinetic rate is proportional to the number of free binding sites (Ghaedi et al. 2013) and can be written as:

$$\frac{\partial [Me^{2+}]_t}{\partial t} = k_1' \left([Me^{2+}]_e - [Me^{2+}]_t \right) \tag{6}$$

$[Me^{2+}]_e$ is the concentration of metal adsorbed at equilibrium, $[Me^{2+}]_t$ is the concentration of metal adsorbed at time t and k_1' (min^{-1}) is the pseudo-first adsorption rate constant. This adsorption rate constant and $[Me^{2+}]_e$ can be computed experimentally from the slope and intercept of the linear plot of ln ($[Me^{2+}]_e - [Me^{2+}]_t$) versus time ($min^{-1}$).

2.2.2 Second-order kinetic model

Second-order kinetic equation (Blanchard et al. 1984) in terms of adsorption has been used frequently (Ho et al. 2000). This model assumes that the rate-limiting step is most likely to involve chemical interactions leading to binding of the ions to the surface by bonding as strong as covalent bonding (Gupta and Bhattacharyya 2011). This model can be expressed as Chojnacka (2010):

$$\frac{\partial[Me^{2+}]_t}{\partial t} = k_2\left(\left[Me^{2+}\right]_e - \left[Me^{2+}\right]_t\right)^2 \quad (7)$$

where k_2 (g mg^{-1} min^{-1}) is the second-order adsorption rate constant. Experimentally, the plot of (t/[Me^{2+}]$_t$) versus time (min^{-1}) results in 1//[Me^{2+}]$_t$ = slope and 1/k_2[Me^{2+}]$_e$ = the intercept.

The pseudo-first kinetic model has been applied to the adsorption of Cd(II) onto *Hypnea valentiae* and *Mastocarpus stellatus* (Rathinam et al. 2010; Herrero et al. 2011). The second-order rate constant model has been applied to the adsorption of Cu(II), Cd(II), Pb(II) and Ni(II) on algae such as: *Ulva fasciata*, *Sargassum* sp. (Karthikeyan et al. 2007), *Laminaria*, *Durvillaea*, *Eckloniaand*, *Homostra* (Figueira et al. 2000), *Bifurcaria bifurcate*, *Saccorhiza polyschides*, *Ascophyllum nodosum*, *Laminaria ochroleuca*, *Pelvetia caniculate* (Lodeiro et al. 2005), *Oedogonium* sp. (Gupta and Rastogi 2008), *Fucus vesiculosus* (Mata et al. 2008), *Cystoseira indica*, *Nizmuddinia zanardini*, *Sargassum glaucescens* and *Padina australis* (Pahlavanzadeh et al. 2010).

2.3 Bioadsorption as a Function of pH

The effect of pH in terms of bioadsorption is based on the binding of metals onto the cell surfaces due to the negatively charged functional groups that form the cell wall. Despite the number of biosorbents, the composition of their cell walls is, as indicated above, very similar. Several authors (Yee and Fein 2001; Fein et al. 2001; Borrok and Fein 2004; González et al. 2010) proposed the principle of ''*universal adsorption-edge*'' developed earlier for heterotrophic bacteria and their consortia and soil bacteria and later used for the bioadsorption capacities of bryophytes (mosses; González and Pokrovsky (2014)). The adsorption of metal cations on the cell surface increases with the pH (Fig. 2), as the available sites to bind metals are fully protonated at low pH. However, this mechanism is only valid for cations (Naja and Volesky 2010). Another explanation considers that pH has a key control over the solubility of heavy metals in solution because it reduces the concentration of OH^- and CO_3^{2-} (Millero et al. 2009).

According to with the LPM (Martinez and Ferris 2001; Martinez et al. 2002; Martinez et al. 2004; Pokrovsky et al. 2008a; González et al. 2010), the pH-edge can be expressed, within this formalism by $K_{s,j}$, as the reaction between metal in solution and available surface sites:

$$Me^{2+} + B_jH^0 \rightarrow^{K_{s,j}} MeB_j^+ + H^+ \quad (8)$$

B_j represents a surface reactive site and $K_{s,j}$ is the apparent concentration equilibrium constant conditional on ionic strength. For a jth deprotonated binding sites at the ith pH values, $K_{s,j}$ is as follows:

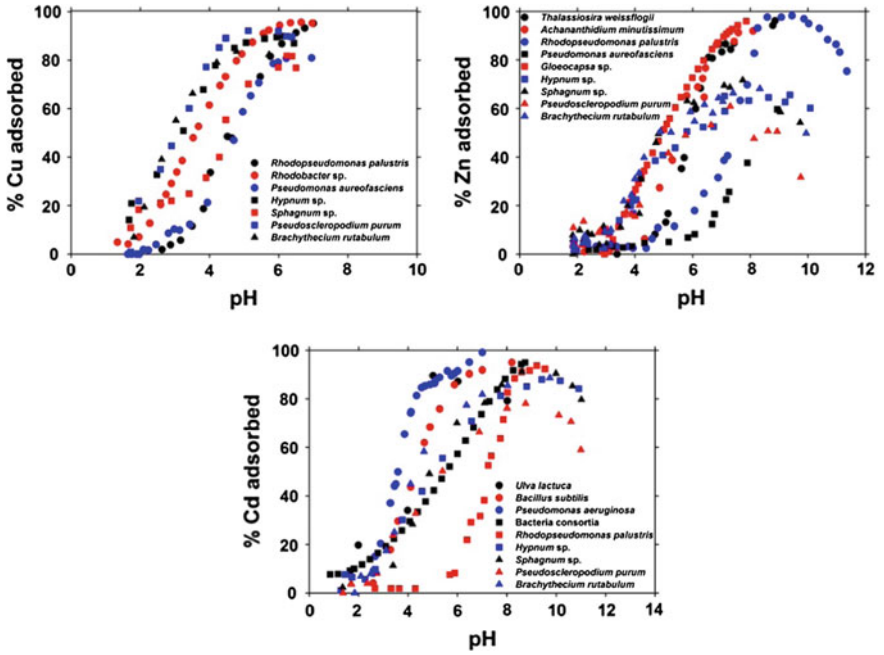

Fig. 2 Adsorption of Cu(II), Zn(II) and Cd(II) on several biosorbents. *Rhodopseudomonas palustris* (4 g L^{-1}; Pokrovsky et al. (2008b)); *Rhodobacter* sp. (4 g L^{-1}; Pokrovsky et al. (2008b)), *Pseudomonas aureofasciens* (4 g L^{-1}; González et al. (2010); Drozdova et al. (2014)); *Hypnum* sp., *Sphagnum* sp., *Pseudoscleropodium purum* and *Brachythecium rutabulum* (1 g L^{-1}; González and Pokrovsky (2014)), *Thalassiosira weissflogii*, *Achananthidium minutissimum* (35 and 25 g L^{-1}; Gelabert et al. (2006)), *Ulva lactuca* (20 g L^{-1}; Sarı and Tuzen (2008)), *Bacillus subtilis* (1 g L^{-1}; Fowle and Fein (2000)); Bacteria consortia (1 g L^{-1}; Borrok and Fein (2004)), *Gloeocapsa* sp. (4 g L^{-1}; Pokrovsky et al. (2008a)), *Pseudomonas aeruginosa* (1 g L^{-1}; Yee and Fein (2001))

$$K_{s,j} = \frac{[MeB_j^+]_i [H^+]_{meas,i}}{[Me^{2+}]_{meas,i} [B_j H^0]_i} \tag{9}$$

where $K_{s,j}$ is a function of experimentally determined proton and metal concentration ($[H^+]_{meas,i}$ and $[Me^{2+}]_{meas,i}$) and the amount of Me^{2+} bound to the jth site at the ith pH value ($[MeB_j^+]_i$).

Figure 2 shows the bioadsorption of Cu(II), Zn(II) and Cd(II) on different biosorbents such as bacteria, marine microalgae, algae, soil bacteria and bryophytes. This wide comparison demonstrates that the universal bio-surface pH-edge occurs for different organisms, probably only due to the composition of the cell wall. Bryophytes and bacteria are the most interesting biosorbents, capable of adsorbing the same amount of metal at a factor of 4–35 lower biomass concentration compared to other sorbents.

2.4 Bioadsorption as a Function of Metal Concentration in Solution

The bioadsorption capacity of a specific biosorbent can be studied as a function of metal cations in solution. The most common bioadsorption equilibrium isotherms are the Langmuir isotherms or Freundlich isotherms, but there are also other types of isotherm as described below.

2.4.1 Langmuir Isotherm

This isotherm has a surface with homogeneous binding sites. A monolayer adsorption occurs on the surface, equivalent adsorption energy and no interaction between adsorbed species (Langmuir 1918; Gupta and Rastogi 2008). In addition, when the binding sites are saturated, no more metal can be adsorbed to these sites. Accordingly, with increasing metal concentration, the surface will reach a saturation point at which the maximum adsorption capacity is achieved (Farhan et al. 2013). The Langmuir isotherm can be expressed as:

$$\frac{[Me^{2+}]_{aq}}{[Me^{2+}]_{ads}} = \frac{1}{K_L q_{max}} + \frac{[Me^{2+}]_{aq}}{q_{max}} \tag{10}$$

$[Me^{2+}]_{aq}$ (mgL^{-1}) is the concentration of aqueous metal, $[Me^{2+}]_{ads}$ ($mg\ g^{-1}$) is the concentration of metal adsorbed onto the biosorbent, K_L is the Langmuir constant related to the energy of adsorption and q_{max} is the maximum adsorption capacity. The q_{max} ($mg\ g^{-1}$) and K_L (Lmg^{-1}) can be determined from the linearization via $[Me^{2+}]_{aq}/[Me^{2+}]_{ads}$ versus $[Me^{2+}]_{aq}$.

2.4.2 Freundlich Isotherm

The Freundlich isotherm considers adsorption on heterogeneous surfaces with the interaction between adsorbed metals (Freundlich 1906). The Freundlich isotherm can be expressed as:

$$\log[Me^{2+}]_{ads} = \log k_F + \left(\frac{1}{n}\right) \log[Me^{2+}]_{aq} \tag{11}$$

where k_F and n are Freundlich constants characteristic of each biosorbent that give us information about the adsorption capacity and adsorption intensity, respectively. When $n = 1$, the adsorption is linear, when $n < 1$ the adsorption is a chemical process, and when $n > 1$, the adsorption is a physical process (Farhan et al. 2013). This equation is exponential and should only be used in the low to intermediate

concentration ranges. The Freundlich equilibrium constants can be computed graphically from the plot of log $[Me^{2+}]_{ads}$ versus log $[Me^{2+}]_{aq}$.

2.4.3 Sips Isotherm

Sips (1948) proposed an empirical isotherm equation:

$$[Me^{2+}]_{aq} = \frac{K_s[Me^{2+}]_{ads}^{ns}}{1 + a_s[Me^{2+}]_{ads}^{ns}} \tag{12}$$

where K_s is the Sips constant (L mg^{-1}), a_s is the affinity coefficient (L mg^{-1}) and n_s is the heterogeneity coefficient.

2.4.4 Redlich–Peterson Isotherm

The Redlich–Peterson isotherm (Redlich and Peterson 1959) incorporates 3 parameters enabling its application in homogeneous and heterogeneous systems (Redlich and Peterson 1959; Padmavathy 2008). This isotherm can be expressed as:

$$\ln\left[\left(\frac{A[Me^{2+}]_{aq}}{[Me^{2+}]_{ads}}\right) - 1\right] = g\ln[Me^{2+}]_{aq} + \ln B \tag{13}$$

A, B and g ($0 < g < 1$) are the three isotherm constants. This isotherm will be the Freundlich isotherm when the concentration of aqueous metal is high. When $g = 1$, the Redlich–Peterson isotherm is equal to the Langmuir isotherm.

2.4.5 Tóth Isotherm

Toth (1971) suggested this isotherm, deriving it from the potential theory. It is applied for bioadsorption on heterogeneous biosorbents. This isotherm can be expressed as follows:

$$[Me^{2+}]_{ads} = q_{max}\frac{b_T[Me^{2+}]_{aq}}{\left[1 + (b_T[Me^{2+}]_{aq})^{1/n_T}\right]^{n_T}} \tag{14}$$

b_T and n_T are constants. This isotherm is reduced to the Langmuir isotherm when $n_T = 1$.

2.4.6 Frumkin Isotherm

The Frumkin isotherm takes the possible interaction between the bioadsorbed species into account (Grchev et al. 1991). This isotherm is expressed as a linear equation according to:

$$ln\left[\left(\frac{[Me^{2+}]_{ads}}{Q_F - [Me^{2+}]_{ads}}\right)\frac{1}{[Me^{2+}]_{aq}}\right] = \ln K_F + 2f\frac{[Me^{2+}]_{ads}}{Q_F} \quad (15)$$

where Q_F is the theoretical monolayer saturation capacity, f is the interaction coefficient and K_F is the equilibrium constant. This expression can be reduced to the Langmuir isotherm when f = 0.

2.4.7 Temkin Isotherm

Temkin and Pyzhev (1940) proposed this isotherm assuming an equal distribution of binding energies over the different binding sites on the surface of the biosorbent, and assuming that the heat of adsorption decreases linearly with the increase of the adsorbent. The linear form of the Temkin isotherm is:

$$[Me^{2+}]_{ads} = \frac{RT}{b}\ln\left(A[Me^{2+}]_{aq}\right) \quad (16)$$

where R is the universal gas constant (8.314 J mol^{-1} K^{-1}), T is the temperature (Kelvin) and B the heat of adsorption. A is the equilibrium binding constant or Temkin isotherm constant.

As we mentioned before, the mathematical approach LPM (Martinez and Ferris 2001; Martinez et al. 2002, 2004) has been extensively used in order to fit the metal

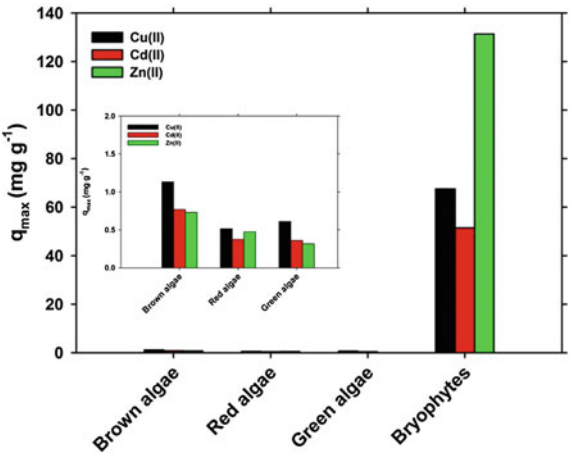

Fig. 3 Maximum adsorption capacity (q_{max}) for brown, green and red algae (see references in Table 1)

Table 1 Maximum adsorption capacity (q_{max}) measured via adsorption of metal ions onto cell surfaces for several biosorbents, at constant pH. Modified from He and Chen (2014)

Brown Algae				
Metal ions	Species	pH	q_{max} (mg g^{-1})	References
Pb(II)	*Ascophyllum nodosum*	3.5	1.31	Holan and Volesky (1994)
	Ascophyllum nodosum	3.0	0.86	Romera et al. (2007)
	Fucus spiralis	3.0	0.98	Romera et al. (2007)
	Fucus vesiculosus	3.5	1.11	Holan and Volesky (1994)
	Fucus vesiculosus	5.0	1.02	Mata et al. (2008)
	Padina pavonia	4.5	1.04	Jalali et al. (2002)
	Padina sp.	5.0	1.25	Sheng et al. (2004)
	Sargassum hystrix	4.5	1.37	Jalali et al. (2002)
	Sargassum natans	3.5	1.22	Holan and Volesky (1994)
	Sargassum natans	4.5	1.14	Jalali et al. (2002)
	Sargassum sp.	5.0	1.16	Sheng et al. (2004)
	Sargassum vulgare	3.5	1.10	Holan and Volesky (1994)
Cu(II)	*Ascophyllum nodosum*	4.0	0.91	Romera et al. (2007)
	Fucus serratus	5.5	1.60	Ahmady-Asbchin et al. (2008)
	Fucus spiralis	4.0	1.10	Romera et al. (2007)
	Fucus vesiculosus	5.0	1.66	Mata et al. (2008)
	Padina sp.	5.0	1.14	Sheng et al. (2004)
	Sargassum filipendula	4.5	0.89	Davis et al. (2000)
		4.5	1.32	Kleinübing et al. (2011)
	Sargassum fluitans	4.5	0.80	Davis et al. (2000)
	Sargassum sp.	5.0	0.99	Sheng et al. (2004)
		5.5	1.13	Karthikeyan et al. (2007)
	Sargassum vulgarie	4.5	0.93	Davis et al. (2000)
Cd(II)	*Ascophyllum nodosum*	6.0	0.78	Romera et al. (2007)
		4.5	0.70	Lodeiro et al. (2005)
	Bifurcaria bifurcate	4.5	0.65	Lodeiro et al. (2005)
	Fucus spiralis	6.0	1.02	Romera et al. (2007)
	Fucus vesiculosus	6.0	0.96	Mata et al. (2008)
	Laminaria ochroleuca	4.5	0.56	Lodeiro et al. (2005)
	Macrocystis pyrifera	3.0	0.89	Cazón et al. (2012)
	Padina sp.	5.5	0.75	Sheng et al. (2004)
	Padina tetrastomatica	5.0	0.53	Hashim and Chu (2004)
	Pelvetia caniculata	4.5	0.66	Lodeiro et al. (2005)
	Saccorhiza polyschides	4.5	0.84	Lodeiro et al. (2005)
	Sargassum baccularia	5.0	0.74	Hashim and Chu (2004)
	Sargassum filipendula	4.5	0.66	Davis et al. (2000)
		5.0	1.17	Luna et al. (2010)

(continued)

Table 1 (continued)

Brown Algae				
Metal ions	Species	pH	q_{max} (mg g^{-1})	References
	Sargassum fluitans	4.5	0.71	Davis et al. (2000)
	Sargassum muticum	4.5	0.68	Davis et al. (2000)
	Sargassum siliquosum	5.0	0.73	Hashim and Chu (2004)
	Sargassum sp.	4.5	0.78	Davis et al. (2000)
		5.5	0.76	Sheng et al. (2004)
	Sargassum vulgarie	4.5	0.79	Davis et al. (2000)
Zn(II)	*Ascophyllum nodosum*	6.0	0.64	Romera et al. (2007)
	Fucus spiralis	6.0	0.81	Romera et al. (2007)
	Macrocystis pyrifera	4.0	0.91	Cazón et al. (2012)
	Padina sp.	5.5	0.81	Sheng et al. (2004)
	Sargassum filipendula	5.0	0.71	Luna et al. (2010)
	Sargassum sp.	5.5	0.50	Sheng et al. (2004)
Ni(II)	*Ascophyllum nodosum*	3.5	0.69	Holan and Volesky (1994)
	Ascophyllum nodosum	6.0	0.73	Romera et al. (2007)
	Cystoseria indica	6.0	0.85	Pahlavanzadeh et al. (2010)
	Fucus spiralis	6.0	0.85	Romera et al. (2007)
	Fucus vesiculosus	3.5	0.39	Holan and Volesky (1994)
	Nizmuddinia zanardini	6.0	0.94	Pahlavanzadeh et al. (2010)
	Padina australis	6.0	0.46	Pahlavanzadeh et al. (2010)
	Padina sp.	5.5	0.63	Sheng et al. (2004)
	Sargassum filipendula	4.5	1.07	Kleinübing et al. (2011)
	Sargassum fluitans	3.5	0.75	Holan and Volesky (1994)
	Sargassum glaucescensand	6.0	0.94	Pahlavanzadeh et al. (2010)
	Sargassum natans	3.5	0.41	Holan and Volesky (1994)
	Sargassum sp.	5.5	0.61	Sheng et al. (2004)
	Sargassum vulgare	3.5	0.09	Holan and Volesky (1994)
Green algae				
Metal ions	Species	pH	q_{max} (mg g^{-1})	References
Pb(II)	*Caulerpa lentillifera*	5.0	0.13	Kleinübing et al. (2011)
	Cladophora glomerata	4.5	0.35	Jalali et al. (2002)
	Cladophora sp.	5.0	0.22	Lee and Chang (2011)
	Codium vermilara	5.0	0.30	Romera et al. (2007)
	Spirogyra insignis	5.0	0.24	Romera et al. (2007)
	Spirogyra neglecta	5.0	0.56	Singh et al. (2007)
	Spirogyra sp.	5.0	0.43	Lee and Chang (2011)
	Ulva lactuca	4.5	0.61	Jalali et al. (2002)
	Ulva sp.	5.0	1.46	Sheng et al. (2004)

(continued)

Table 1 (continued)

Brown Algae				
Metal ions	Species	pH	q_{max} (mg g^{-1})	References
Cu(II)	*Caulerpa lentillifera*	5.0	0.08	Pavasant et al. (2006)
	Cladophora sp.	5.0	0.23	Lee and Chang (2011)
	Codium vermilara	5.0	0.26	Romera et al. (2007)
	Spirogyra insignis	4.0	0.30	Romera et al. (2007)
	Spirogyra neglecta	4.5	1.80	Singh et al. (2007)
	Spirogyra sp.	5.0	0.60	Lee and Chang (2011)
	Spirogyra sp.	5.0	0.53	Rajfur et al. (2012)
	Ulva fasciata	5.5	1.14	Karthikeyan et al. (2007)
	Ulva fasciata	5.0	0.42	Karthikeyan et al. (2007)
	Ulva sp.	5.0	0.75	Sheng et al. (2004)
Cd(II)	*Caulerpa lentillifera*	5.0	0.04	Pavasant et al. (2006)
	Chaetomorpha linum	5.0	0.48	Hashim and Chu (2004)
	Codium vermilara	6.0	0.19	Romera et al. (2007)
	Oedogonium sp.	5.0	0.79	Gupta and Rastogi (2008)
	Spirogyra insignis	6.0	0.20	Romera et al. (2007)
	Ulva lactuca	5.0	0.25	Sarı and Tuzen (2008)
	Ulva sp.	5.5	0.58	Sheng et al. (2004)
Zn(II)	*Caulerpa lentillifera*	5.0	0.04	Pavasant et al. (2006)
	Codium vermilara	6.0	0.36	Romera et al. (2007)
	Spirogyra insignis	6.0	0.32	Romera et al. (2007)
	Ulva sp.	5.5	0.54	Sheng et al. (2004)
Ni(II)	*Codium vermilara*	6.0	0.22	Romera et al. (2007)
	Spirogyra insignis	6.0	0.29	Romera et al. (2007)
	Ulva lactuca	4.5	1.14	Zakhama et al. (2011)
	Ulva sp.	5.5	0.29	Sheng et al. (2004)
Red algae				
Metal ions	Species	pH	q_{max} (mg g^{-1})	References
Pb(II)	*Asparagopsis armata*	4.0	0.30	Romera et al. (2007)
	Chondrus crispus	4.0	0.98	Romera et al. (2007)
	Corallina mediterranea	5.0	0.31	Ibrahim (2011)
	Galaxaura oblongata	5.0	0.42	Ibrahim (2011)
	Gracilaria canaliculata	4.5	0.20	Jalali et al. (2002)
	Gracilaria corticata	4.5	0.26	Jalali et al. (2002)
	Gracillaria sp.	5.0	0.45	Sheng et al. (2004)
	Jania rubens	5.0	0.14	Ibrahim (2011)
	Polysiphonia violacea	4.5	0.49	Jalali et al. (2002)
	Pterocladia capillacea	5.0	0.16	Ibrahim (2011)

(continued)

Table 1 (continued)

Brown Algae				
Metal ions	Species	pH	q_{max} (mg g^{-1})	References
Cu(II)	*Asparagopsis armata*	5.0	0.33	Romera et al. (2007)
	Chondrus crispus	4.0	0.63	Romera et al. (2007)
	Gelidium sp.	5.3	0.51	Vilar et al. (2008)
	Gracillaria sp.	5.0	0.59	Sheng et al. (2004)
Cd(II)	*Asparagopsis armata*	6.0	0.28	Romera et al. (2007)
	Ceramium virgatum	5.0	0.35	Sarı and Tuzen (2008)
	Chondrus crispus	6.0	0.66	Romera et al. (2007)
	Corallina mediterranea	5.0	0.57	Ibrahim (2011)
	Galaxaura oblongata	5.0	0.76	Ibrahim (2011)
	Gracilaria changii	5.0	0.23	Hashim and Chu (2004)
	Gracilaria edulis	5.0	0.24	Hashim and Chu (2004)
	Gracilaria salicornia	5.0	0.16	Hashim and Chu (2004)
	Gracillaria sp.	5.5	0.30	Sheng et al. (2004)
	Hypnea valentiae	6.0	0.15	Rathinam et al. (2010)
	Jania rubens	5.0	0.27	Ibrahim (2011)
	Mastocarpus stellatus	6.0	0.59	Herrero et al. (2011)
	Pterocladia capillacea	5.0	0.29	Ibrahim (2011)
Zn(II)	*Asparagopsis armata*	6.0	0.33	Romera et al. (2007)
	Chondrus crispus	6.0	0.69	Romera et al. (2007)
	Gracillaria sp.	5.5	0.40	Sheng et al. (2004)
Ni(II)	*Asparagopsis armata*	6.0	0.29	Romera et al. (2007)
	Chondrus crispus	6.0	0.63	Sheng et al. (2004)
	Gracillaria sp.	5.5	0.28	Sheng et al. (2004)
Others (Microalgae, Soil bacteria, Bacteria, Bryophytes)				
Metal ions	Species	pH	q_{max} (mg g^{-1})	References
Pb(II)	*Australian Marine algae DP95Ca*	5.0	320.85	Matheickal and Yu (1999)
	Australian Marine algae ER95Ca	5.0	260.82	Matheickal and Yu (1999)
	Bacillus cereus	5.5	36.71	Pan et al. (2007)
	Rhodococcus opacus	5.0	94.19	Bueno et al. (2008)
	Hypnum sp.	6.5	312.66	González and Pokrovsky (2014)
	Sphagnum sp.	6.5	229.78	González and Pokrovsky (2014)
	Pseudoscleropodium purum	6.5	182.34	González and Pokrovsky (2014)
	Brachythecium rutabulum	6.5	530.43	González and Pokrovsky (2014)

(continued)

Table 1 (continued)

Brown Algae				
Metal ions	Species	pH	q_{max} (mg g^{-1})	References
Cu(II)	*Australian Marine algae DP95Ca*	5.0	82.55	Matheickal and Yu (1999)
	Australian Marine algae ER95Ca	5.0	70.49	Matheickal and Yu (1999)
	Bacillus cereus	5.5	50.32	Pan et al. (2007)
	Hypnum sp.	5.5	63.16	González and Pokrovsky (2014)
	Sphagnum sp.	5.5	81.85	González and Pokrovsky (2014)
	Pseudoscleropodium purum	5.5	86.17	González and Pokrovsky (2014)
	Brachythecium rutabulum	5.5	49.69	González and Pokrovsky (2014)
	Sphagnum denticulatum	5.5	79.44	González et al. (2016c)
	Sphagnum palustre	5.5	42.58	González et al. (2016b)
		5.5	69.27	González et al. (2016b)
Cd(II)	*Hypnum* sp.	6.5	35.75	González and Pokrovsky (2014)
	Sphagnum sp.	6.5	81.95	González and Pokrovsky (2014)
	Pseudoscleropodium purum	6.5	29.56	González and Pokrovsky (2014)
	Brachythecium rutabulum	6.5	51.26	González and Pokrovsky (2014)
Zn(II)	*Achananthidium minutissimum*	6.2	0.36	Gelabert et al. (2006)
	Hypnum sp.	6.8	45.90	González and Pokrovsky (2014)
	Sphagnum sp.	6.8	60.74	González and Pokrovsky (2014)
	Pseudoscleropodium purum	6.8	58.91	González and Pokrovsky (2014)
	Brachythecium rutabulum	6.8	68.58	González and Pokrovsky (2014)
	Skeletonema costatum	7.2	2.62	Gelabert et al. (2006)
	Sphagnum denticulatum	6.5	60.80	González et al. (2016c)
	Sphagnum palustre	6.5	253.02	González et al. (2016c)
		6.5	300.75	González et al. (2016c)
	Thalassiosira weissflogii	7.5	0.131	Gelabert et al. (2006)
Ni(II)	*Chlorella vulgaris*	4.5	86.60	Aksu and Dönmez (2006)
	Hypnum sp.	5.6	5.22	González and Pokrovsky (2014)

(continued)

Table 1 (continued)

Brown Algae				
Metal ions	Species	pH	q_{max} (mg g^{-1})	References
	Sphagnum sp.	5.6	6.28	González and Pokrovsky (2014)
	Pseudoscleropodium purum	5.6	3.64	González and Pokrovsky (2014)
	Brachythecium rutabulum	5.6	4.17	González and Pokrovsky (2014)

adsorbed onto the cell wall at fixed pH (González et al. 2010; González and Pokrovsky 2014; González et al. 2016c; Pokrovsky et al. 2008a). In this sense, in the case of the Langmuir isotherm adsorption (at fixed pH) the reaction between metal and binding sites can be expressed as:

$$Me^{2+} + B_j^- \rightarrow^{K_{m,j}} MeB_j^+ \tag{17}$$

where B_j is the specific surface functional group and $K_{m,j}$ is the apparent metal-ligand binding constant conditional on ionic strength. At *jth* deprotonated functional group and fixed pH value is written as follows:

$$K_{m,j} = \frac{\left[MeB_j^+\right]}{[Me^{2+}]_{meas,i}[B_j]_i} \tag{18}$$

In addition, $K_{m,j}$ is a function of experimental metal concentration ($[Me^{2+}]_{meas,i}$) and of the amount of Me^{2+} bound to the j*th* site as a function of increasing biomass and at fixed pH value ($[MeB_j^+]_i$). In the same way as the above treatment, the available binding sites are computed and assigned to a fixed $pK_{m,j}$ grid.

Note that the values of $K_{s,j}$ and $K_{m,j}$ computed are not directly comparable because K_s is a function of K_m ($K_s = K_m \cdot K_a$), where K_a is the acidity constant for a specific functional group on the biosorbent surface.

Figure 3 represents the q_{max} (mg g^{-1}) for the most interesting groups of biosorbents (Table 1.). Bryophytes are able to adsorb > 60-fold Cu(II), > 50-fold Cd(II) and > 200-fold Zn(II) compared to brown, red and green algae, respectively. It is important to remark that the bryophytes here include natural and produced under laboratory conditions (González et al. 2016c). In this regard, the comparison of the biosorption capacity of algae, brown algae are the most interesting ones because of the composition of their cell wall, with high levels of carboxyl and hydroxyl functional groups (Davis et al. 2003; Vieira and Volesky 2000).

Table 1 represents a compilation of the biosorption capacities (q_{max}) of several biosorbents (algae, bacteria, soil bacteria and bryophytes).

3 Commercial Applications

Adsorption of heavy metals on biosorbents has been mainly investigated at laboratory scale, as can be concluded from the references cited in this chapter. Only a few companies and laboratories have been involved in transferring the bioadsorption to industrial scale. The future goes through this transformation and this is the only way to obtain satisfactory results to solve the problem of pollution with heavy metals, especially in water bodies.

The USA and Canada were the first countries to carry out bioadsorption processes at industrial scale, accepted by EPA (EPA/540/S5-90/005). The positive results obtained by these experiences motivated the scientists to continue, though timidly, the application at this level, although we need to improve our knowledge about bioadsorption processes and biosorbents to obtain satisfactory and long-term results. On the other hand, future research should address improving our knowledge about the adsorption capacity of biosorbents in multi-heavy metal solutions, as the metal interaction in solution can affect the redox chemistry of these metals (i.e. copper–iron interactions; González et al. (2016b) or this interaction can affect the binding capacity (González-Dávila et al. 1995; Bueno et al. 2008).

Different commercial biosorbents and their characteristics are presented here.

(a) **BIO-FIX** is commercialized by the US Bureau of Mines (Golden, Co, USA) to remove heavy metals during the wastewater treatment. Bio-fix is a porous polymeric bead produced with non-living biomaterial: peat moss, algae, biological polymers and other materials with high metal affinity (Jeffers et al. 1993). These beads have an excellent handling and they have been satisfactory used for Cu(II), Cd(II), Zn(II) and Pb(II). In addition, this material can be reused due to the high reversible characteristics after acid solution treatment.

(b) **AMT-BIOCLAIM** has been developed by Advanced Mineral Technologies, Inc. (AMT) via bacterial fermentation processes (Brierley 1985, 1990). They used *Bacillus subtilis* as main bacteria because it is one of the most studied bacteria in terms of bioadsorption. This material also presents high recovery rates of heavy metal, being ~99% for Ag, Cd, Cu, Pb and Zn.

(c) **ALGASORB™** was produced by Bio-Recovery System, Inc. (USA). It consists in Chlorella vulgaris immobilized in silica gel polymer matrix. It can be used for a wide range of heavy metal concentrations (1–100 mg g^{-1}). The material is immobilized in silica gel polymer for protection of the cells and to be able to form a sorbent that can be added in columns. ALGASORB™ can be reused after treatment with acid–base solutions to remove the adsorbed material.

(d) **BV-SORBEX™** is produced by BV Sorbex, Inc. (Montreal, Canada). This adsorbent contains powder and granules (0.1–3 mm) formed by algae (*Sphaerotilus natans, Ascophyllum nodosum, Halimeda opuntia, Palmyra pomata, Chondrus crispus, Chlorella vulgaris*) (Volesky 2003), and they are able to recover ~99% of metal in solution (available on the BV Sorbex website; http://www.bvsorbex.net).

Acknowledgement Aridane G. González thanks the French 'Agence Nationale de la Recherche' through the 'Laboratoire d'Excellence' LabexMER (ANR-10-LABX-19-01) program, and co-funded by a grant from the French government through the 'Investissements d'Avenir' and the Brittany Region. Oleg S. Pokrovsky thanks the support from BIO-GEO-CLIM grant No 14. B25.31.0001. J. Magdalena Satana-Casiano and Melchor González-Dávila thank the Project CTM2014-52342-P given by the Ministerio de Economia y Competitividad from Spain.

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Wastewater Treatment Using Phototrophic–Heterotrophic Biofilms and Microbial Mats

J. Paniagua-Michel

Abstract Phototrophic and photoheterotrophic microbial biofilms and mats are characterized by its abundant microbial diversity and unique properties in consortia functional populations. Recently, the roles of phototrophic microbial biofilms and mats involving microalgae (diatoms, green algae and cyanobacteria, and other members of bacteria, fungi, and protozoa's) are receiving special attention because of their promissory first results obtained in the sustainable bioremediation of environment-sensitive habitats and effluents. The bioremediation of urban wastewater in coastal-marine environments, petroleum hydrocarbons and heavy metals and metalloid toxicity is a reality. Microbial biofilms and mats also play important bioremediation roles of aquaculture effluents, in the case of Penaeidae shrimp aquaculture effluents, figures of 80–97% removal efficiencies of nitrogen and phosphorous have been reported. Reported figures on the treatment of petroleum compounds achieved removal efficiencies of 25–85%. The uniqueness of many of these properties and processes for phototrophic and heterotrophies microbial biofilms and mats have led to innovative strategies benefiting biofilm-based bioremediation and environmentally safe bioprocesses, such as microbial consortia interactions, quorum sensing, gene exchange and uses of omics technologies. The application in the deep-sea tracking associated to petroleum extraction from shale rock, and in desalination of high salt seawater effluents are technologies based on biofilms and mats already in progress.

Keywords Phototrophs · Heterotrophs · Biofilms · Microbial mats Bioremediation · Wastewater

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B.N. Tripathi and D. Kumar (eds.), *Prospects and Challenges in Algal Biotechnology*, DOI 10.1007/978-981-10-1950-0_9

1 Introduction

In aquatic-marine environments, biofilms are structured assemblages of microorganisms and microalgae (eukaryotic and prokaryotic), viz, diatoms, green algae and cyanobacteria, and other members of bacteria, fungi, and protozoa's (Buhmann et al. 2012). Microbial mats and Biofilms can be also structured as mixed photosynthetic species of algae and cyanobacteria capable to develop photoautotrophic–heterotrophic nutrition that when exposed to light are able to incorporate carbon dioxide and becoming the dominant primary producers to simultaneously generate energy and reduce carbon dioxide (Fig. 1). Hence, organic substrates and oxygen are supplied in the systems through the organic carbon for the development of heterotrophic microorganisms (Kumar et al. 2010; Buhmann et al. 2012). For example, microalgal products from photosynthesis are utilized by heterotrophs as their main source of organic carbon and nitrogen, hence heterotrophs participate in continuous nutrient production (Roeselers et al. 2008). Photoautotrophic biofilms are the only condition that could be compared with microbial mats but not biofilms lacking photosynthetic phototrophic communities. Microbial mats can be applied in many areas and in different uses, viz, bioremediation of wastewater and hydrocarbons from polluted and sensitive environments as well as in aquaculture effluents (Bender and Phillips 2004). Recently, the roles of phototrophic microbial biofilms and mats involving microalgae are receiving special attention because of their promissory first results obtained in the sustainable biotreatment of environmental bioprocesses such as in bioremediation of coastal-marine environments (hydrocarbons and heavy metals) as well as aquaculture effluents.

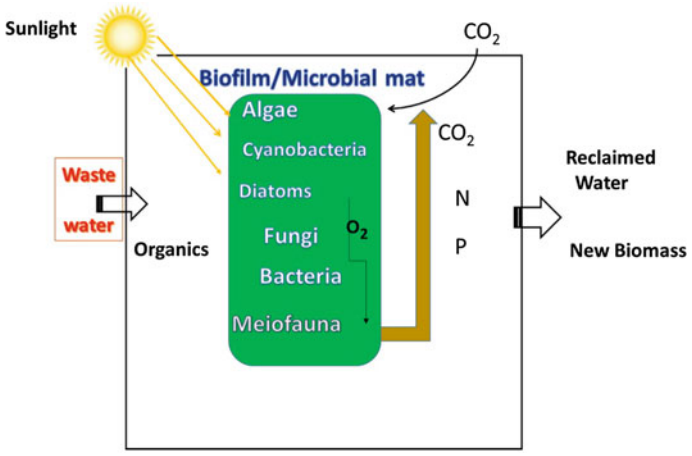

Fig. 1 Microbial-Algal biofilm and their uses in production of reclaimed wastewater through bioremediation

It is well known that coastal states in developing countries are abundant in two classes of waters: seawater and wastewater, which are ideal conditions for the development of biofilms/mats based alternatives. In coastal cities, municipal wastewater treatment plants releases discharge of wastewater into coastal-marine water bodies, most of the time, these water discharges are deficiently treated (Paniagua-Michel et al. 2005). This situation becomes worsened because conventional biological treatment processes typically do not remove total values of nitrogen and phosphorus to the levels needed to protect receiving waters. That is why environmentally safe wastewater treatment alternatives are increasingly being required to reduce pollutants concentration to safe levels (Munoz and Guieysse 2006; Ahmad et al. 2013). Bioremediation is considered an important biotechnology of the century to mitigate, destroy, or restore several types of pollutants of an environmentally altered ecosystem in a sustainable fashion at relatively low cost (Ward 2004). In these processes, the vast metabolic diversity of the living biomes are responsible to break down a variety of pollutants and to achieve a waste reduction by enhancing the biodegradation and removal of processes that occur in nature or under ex-situ technologies.

Among the present biological alternatives of this kind of effluents and environments, bioremediation is an actual and promissory biological treatment process utilizing algae, or microbial consortia of cyanobacteria and bacteria. Microalgae in microbial mats are preferred because may contribute with bacteria in remediating heavy metals, oils, as well as nutrients, and represent an eco-friendly contribution to the environment. Hence, microalgae are an attractive and potential candidate as microbiome for bioindustrial applications and bioprocesses (Rawat et al. 2011). Bioremediation by microalgae is a generally desirable biotechnology in many aspects and different applications because of the versatile roles displayed by bioremediators organisms, mainly its feasibility to generate biomass for biofuel production simultaneously to carbon sequestration (Mulbry et al. 2008). Moreover, because microalgae applied to wastewater remediation does not release any secondary pollution, places the biotreatment among the most environmentally compatible process considering that the biomass produced and continuously regenerated is based on the recycling of nutrients from wastewater (Munoz and Guieysse 2006; Mulbry et al. 2008). In this context, photoautotrophic and photoheterotrophic biofilms and microbial mats are relevant components for the biotreatment of environmentally impacted ecosystems. Hence, biofilms and microbial mats acquire planetary and exobiological dimensions, which explain their global importance.

The objective of this chapter is to analyze actual developments as well as the potential applications of photoautotrophic and heterotrophic biofilms, and microbial mats, mostly composed by cyanobacteria and eukaryotic microalgae. Their role in associated processes with the bioremediation of wastewater effluents, as well as in the removal and bioremediation of heavy metals, petroleum hydrocarbons, and aquaculture effluents will be also analyzed and discussed in this chapter.

2 Defining Microalgal Biofilms and Mats

Consortia of cells in multicellular arrangements or biofilms have been intensively surveyed and considered as the primary growth model of microbial life (Horemans et al. 2015).

Microbial-based biotechnologies, basically, relies on the availability of Biofilms, and microbial mats- based bioremediation systems. For that reason, the bioprocesses associated to these microbial structures, are clever to improve bioremediation of polluted wastewater effluents and environments. Most of the technologies based on these microbial processes, viz, bioreactors (aerobic and anaerobic), and rotating disk contactors, still remains challenging worldwide. The functionality of biofilms is usually associated with a matrix of extracellular polymeric substances, which exert strong correlation to microbial interactions within the biofilm and respective gene exchange (Franco et al. 2006). The uniqueness of the properties and processes associated to biofilms, represent important potentials for the enhancement of the biofilm-based bioremediation (Horemans et al. 2015).

In nature, and almost as a general rule, photoautotrophic and photoheterotrophic biofilms are communities composed mainly of microorganism, such as bacteria, fungi, and protozoa, although some authors include algae and specific meiofauna in aquatic environments (Cooksey 1992). On the other side, microbial mats, are composed mainly of photosynthetic eukaryotes and prokaryotes as well as bacteria. Thus, their source of energy dependent on photosynthetic primary productivity and represent the main distinction between microbial mats and other biofilms.

The algal colonies members of both structures and respective filaments are typically embedded in a matrix of exopolysaccharides. Some authors exclude algae in some biofilms definition, which automatically invalidate the definition and or synonymy with microbial mats. Microbial mats are integral ecosystems in which **different** organisms perform different roles (producer, consumer, and decomposer) in the ecosystem. In nature, microbial mats are multilayered polymicrobial communities mainly stratified vertically and laminated in a consortium of bacteria and photoautotrophic and heterotrophic microalgae (Nisbet and Fowler 1999) that obtain their energy from the functional groups that performs photosynthetic primary production (Stal et al. 1985).

That spatial organization undergoes development mostly in sheltered and shallow coastal areas and intertidal zones, as a result of physicochemical gradients. Remarkable similarities to fossil stromatolites can be observed in modern microbial mats built by cyanobacteria. Purple and sometimes green sulfur bacteria are normal components of most cyanobacterial mats. A major problem is the fact that the great majority of present day microbial mats does not form consolidated rock. Microbialmats communities exhibit natural properties to sequester organics and metals from their environment, which place them as an interesting option for the sustainable environmental bioremediation of coastal-marine environments. In principle, depending on the dominant versatility of taxa of bacteria and or algae in biofilms, and mats, when they can exhibit functional interactions leading to new

Table 1 Basis of cellular interactions in microbial biofilms (modified after Van Houten 1992)

Compound	Active and/or Potential involvement in consortia metabolism
	Produced by heterotrophs, electron acceptor for heterotrophs
	Produced by heterotrophs, carbon source for phototrophs
Organic N or C	N may be limiting for both phototrophs or heterotrophs. Carbon could be used either component of the biofilm
Bioactive molecules Allelochemicals	Compounds such as cyclic AMP or chemo attractants may influence biofilm establishment. Vitamins, antibiotics, especially B12 (Algae)
DNA	not likely to be transferred from prokaryote to eukaryote or vice versa, but prokaryote to prokaryote transfer likely

and different useful approaches for bioremediation of wastewaters. When bacteria and microalgae from biofilms and mats are subjected to light illumination, different interactions can be developed, such as syntrophicmutualistic interactions (Sanchez et al. 2006). Thus, a chemical interrelationship between bacteria and algae metabolism is expected to exist, a contrary situation or even a mutual symbiotic condition can be developed.

Some parameters and molecules that influence the molecules at the levels of cell–cell interactions are shown In Table 1.

3 The Role of Bacteria and Microalgae in Wastewater

When compared to conventional physical–chemical methods, biological treatments represents more versatile and environmentally friendly alternative to be used in environmentally sensible environments, such as the coastal-marine environments.

Physical/chemical treatments such as in the conventional wastewater treatment plants are usually costly and generate secondary pollution (Zamora et al. 2007). For instance wastewater effluent production. Wastewater Treatments based on algae offers simultaneously advantages of nutrient recycling, water treatment as well as algal biomass production which can be of industrial interest. That is why the uses and application of microalgae for the treatment of domestic wastewater has emerged recently as a worldwide sustainable biotechnology. The functional role of the different members of the biofilms and mats exert an important enhancement of production of algae biomass, dependent on the consumption of nitrogen and phosphorus.

Generally, mixed populations of strains of microorganisms either in consortia or in co-culture are more successful to thrives in almost any environment and are more efficient in their integral utilization of wastes–nutrients, physiology, and growth,

contrary to individual strains or single species (Subashchandrabose 2011a, b; Brenner et al. 2008).

Other advantages that microalgal populations show in polymicrobial communities, mixed populations, or consortia are: (a) Protection and stability for the members, (b) Ability to protection against toxic pollutants and to share metabolites, (c) Tolerance to environmental fluctuations and to periods of nutrient limitations, (d) Resistance to invasion by other species as well as on the consortia of cyanobacteria/microalgae–bacteria for pollutant degradation.

During the treatment of wastewater, the mineralization of organic pollutants is an important functional process that algal biomass develops to supply oxygen to heterotrophic aerobic bacteria.

Comparatively to biofilms and microbial mats, the use of suspended algae in wastewater treatment systems, generate secondary pollution by algal erosion and washout representing a limitation that may contribute 60–90% of the effluent BOD (Brenner et al. 2008). Hence, in the case of secondary wastewater effluents exposed to environmental conditions, such as enhanced algal productivity by light, attached microorganisms in biofilms of algae and/or bacteria, avoid the fastidious process of biomass separation from water (Subashchandrabose et al. 2011).

But if a more efficient detoxification of organic and inorganic pollutants is desired, the use of consortia of cyanobacteria/microalgae and bacteria can be a more integrated solution for the removal of nutrients from wastewaters than individual microorganisms (Table 2).

In the case of biofilms and microbial mats, photosynthetic cyanobacterial and algal components emit oxygen that represents an important electron acceptor that stimulates heterotrophic bacteria for the degradation of pollutants (Subashchandrabose et al. 2011). But bacteria contribute to the treatment of wastewater, mainly by providing carbon dioxide as well as other stimulatory substances achieving an efficient support for photoautotrophic components of the biofilms and microbial mats. Bacteria performs degradation of organic matter incorporating molecular oxygen produced by algal photosynthesis, while bacterial mineralization generates carbon dioxide which completes the photosynthetic loop. The photosynthetic cycle

Table 2 Typical loading of municipal wastewater (BITION 2005) susceptible to be bioremediated by Microbial biofilms and Mats

Wastewater characteristics	Concentration (mg/L)		
	Strong	Medium	Weak
Suspended solids	350	220	100
Total solids	1200	720	350
Biochemical oxygen Demand (BOD_5)	400	220	110
Chemicaloxygen demand (COD)	1000	500	250
NH_3-N	50	25	12
Totai-N	85	40	20
Organic N	35	15	8
Total P	15	8	4

is completed by carbon dioxide (CO_2) from the bacterial mineralization. Among the properties of the wastewater treatment ponds, the prevailing biological oxygen demand during and after the treatment of the wastewater is a factor that directly depends on the interactions between microalgae and bacteria, either by symbiosis or other sharing functions (Oswald et al. 1953). Nevertheless, the type of microbial associations, such as cooperation, or the competence between autotrophic algae and heterotrophic bacteria are factors that can be dependent on the interrelationship between species, environmental conditions, and the species composition (Subashchandrabose et al. 2011).

For a long time, most of the photosynthetic algal systems were considered autotrophic, but studies of the last decade have revealed that some other nutrients or growth promoting factors are essential, such as certain vitamins mainly biotin, thiamine, and cobalamine (Croft et al. 2006). On the other hand, deficient growth nutrient conditions, as in the case of iron lacking growth conditions can be supplied by bacterial siderophores (Butler 1998). In principle, a symbiotic relationship with bacteria favors algae to obtain vitamin B12 (Croft et al. 2005). Nevertheless, bacterial mineralization in wastewaters provides carbon dioxide to be used in the production of sugars acetate and glycerol through heterotrophic growth by photosynthetic processes of algae and cyanobacteria. These physiological advantages allow algae and cyanobacteria lead to the transformation, degradation, and removal of pollutants in bioremediation technologies (Figs. 1 and 2).

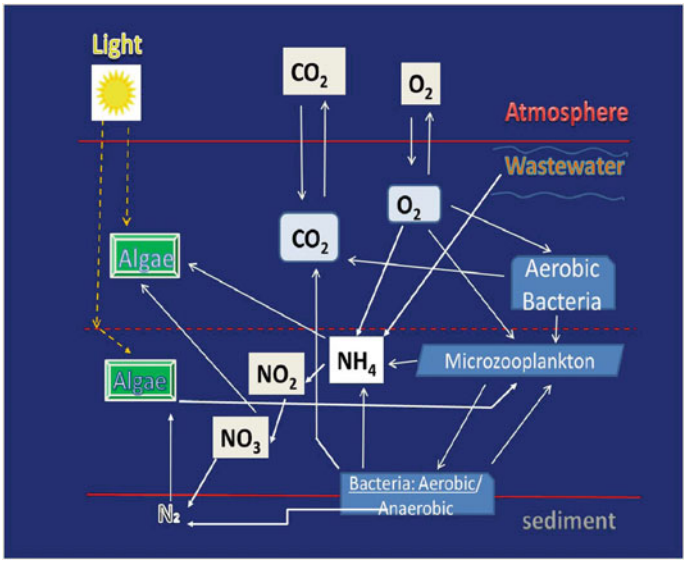

Fig. 2 Interrelationships among polymicrobial communities dominated by microalgae and bacteria in natural and culture ponds and their different interactions with nutrients, oxygen and carbon dioxide (modified after Moriarty 1997)

4 Photobiofilms/Mats for Photobioreactors: Effective Nutrient Removal from Wastewaters

Actually, phototrophic and photoheterotrophic biofilms and mats are cultured and scaled-up in photobioreactors built *ex-profeso* and at the different levels, viz, pilot and industrial scales aiming the removal of pollutants–nutrients from the wastewater effluents. That technology ensures the bioremediation processes to progress toward sustainable production of microalgal biomass to be used in bioindustrial applications, such as value-added metabolites and feedstocks for the production of biofuels. Recently hybrid bioreactors have been developed as efficient systems for wastewater treatment. Recent studies with heterotrophic and autotrophic microorganisms in a hybrid bioreactor reported higher removal 2004 efficiencies when compared to bioreactor systems lacking hybrid components, mainly in NO_3-N (79%), NH_4-N (86%), Total phosphorous (81%), and 82% for total nitrogen (Subashchandrabose et al. 2011; Wu et al. 2011).

The uses of microbial mats immobilized on polymeric substrates were designed as "constructed" or "artificial" by Bender and Phillips (2004). Paniagua-Michel and Garcia (2003) and Zamora et al. (2007) applied artificial microbial mats constructed with isolated microbial consortia for the bioremediation of a municipal wastewater effluent. Analysis of the mat revealed a functional structure dominated by Cyanobacteria genera (Chroococcus sp. and Lyngbya sp), Eukaryote algal cells mainly Nitzschia sp. and β-proteobacteria (Eubacteria), nitrospira and nitrosomonas sp. Excess of nutrients (mainly nitrogen and phosphorus) from the discharged plant effluent enhanced the growth of the different communities forming the microbial mat, which efficiently removed ammonia and allowed the progression of nitrification and mitigation of the main pollutants in the wastewater effluent.

5 Metal Pollutants Removal by Phototrophic Biofilms and Mats

Microalgae, as well as bacteria, have been recognized as efficient heavy metal accumulators from their surrounding environments, which has been utilized in different treatment systems. The use of immobilized algae in biofilms as well as in microbial mats for binding metals is in great part due to the cellular surfaces affinity for metals, which has been applied in the detoxification and recovery of metals from polluted effluents (Greene and Bedell 1990). Carbohydrates and exopolysaccharides are the most abundant component of the cell walls of bacteria, microalgae, and cyanobacteria, which may contain negatively charged (amino, carboxyl, hydroxyl or sulfide) groups (Subashchandrabose et al. 2011). The principle of metal removal from wastewater is based on the relationship between metals and the negatively charged ligands. This bioprocess of metal adsorption by the cell surface can be simultaneously complemented by exopolymeric substances

and exopolysaccharides, by cellular uptake, stored in vacuoles and deposition on the cell surface (Subashchandrabose et al. 2011).

Heavy metals in the wastewater may also inhibit or even arrest the functional role, and photosynthesis of microalgae in biofilms and microbial mats, since in active sites of specific enzymes, metals are able to substitute or even arrest the prosthetic metal atoms.

Moreover, aqueous cations in the pollutants may bind to acidic functional groups of bacterial cell walls affecting basic and functional properties of heavy metals such as speciation, distribution, and mobility (Ginn and Fein 2008).

That is why in environmentally sensitive environments, algae, and bacteria growing in wastewater can be used as an efficient alternative for long-term treatment and removal of metal pollutants.

In the case of the detoxication of uranium from wastewater by algae, recent analysis allows to set up a three-step process: first U the ligands in algal cell walls efficiently remove U (VI), second, the removal of U-algal particulates proceeds from the water column to the sediments. At the end of the process, the heterotrophic bacteria receives nutrient and energy (nitrogen, phosphorous and carbon) from the disintegration of dead algal cells, leading to the final reduction of U(VI) to U(IV) (Kalin et al. 2004; Subashchandrabose et al. 2011).

The mixed populations of Cyanobacterial and bacteria biomass can be used safely to remove heavy metals from wastewaters.

Studies conducted in a biofilter by Loutseti et al. (2009), demonstrated removal efficiencies around 80% of copper and 100% of cadmium with dried mass of mixed culture of microalgae (Scenedesmus sp., Tetraedron sp., Chlorella sp., and Chlorococcus sp.), Pseudoanabaena sp., Leptolyngbya sp., diatoms (Navicula sp., Nitzschia sp., and Cyclotella sp.), and bacteria in a biofilter (Loutseti et al. 2009). Metal tolerance by bacterial and microalgal communities is a process that in most of the cases shows dependence to physiological adaptation, genetic changes or the succession of sensitive species by more tolerant bacteria. The analysis of the role of microbial components associated with wastewater treatment suggested that a correlation can exist between the physiological and genetic structure of bacterial bioremediators, in relation mainly to species composition of algae, and secondarily to the level of metal pollution (Boivin et al. 2007).

6 Role of Microbial Biofilms and Mats in the Biodegradation of Oil Hydrocarbons

(a) Cyanobacterial Mats and biofilms

Bioremediation of oil spills in marine environments by biofilms and microbial mats has been found as a suitable biotechnology (Fig. 3). Hence, preventive actions of the negative environmental and socioeconomic impact of oil and other hydrocarbon

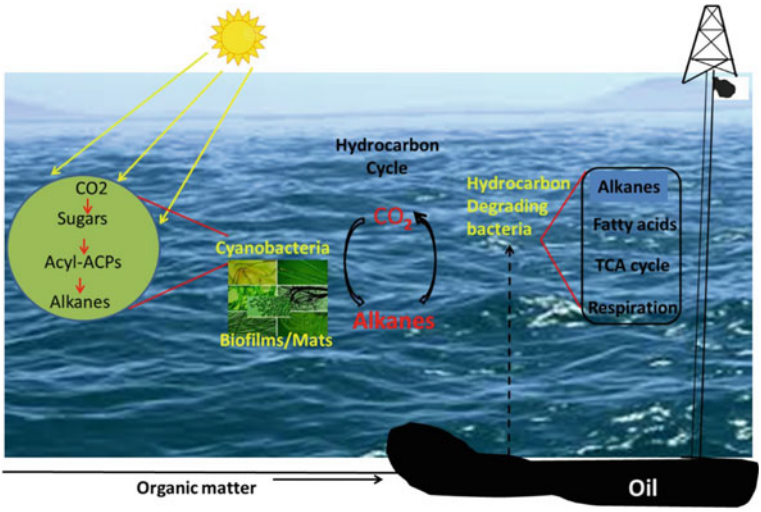

Fig. 3 Fig. Schematic representation of cyanobacterial biofilms and the ocean hydrocarbon cycle. The conversion of CO_2 to alkanes by energy from photosynthesis is indicated in the left by cyanobacterial cells structured in biofilms and or mats and the metabolism of alkanes by the same action by hydrocarbon-degrading bacterial cell (modified after Lea-Smith 2015)

derivatives mainly in industrial activities in the marine environment, such as recreational activities, fisheries, aquaculture and wildlife species (Pazos et al. 2016).

Because coastal-marine environments are highly sensitive to pollutants and their physical and chemical treatment, sustainable and environmental-compatible solutions to these stressors are expected to overcome these difficulties. The wide distribution of biofilms harboring hydrocarbonoclastic bacteria and oil-degrading cyanobacteria contributes to the natural and eco-friendly depuration of contaminated environments with different types of oils and their respective derivatives (Pazos et al. 2016).

Even when such biofilms grow on solid and semisolid substrates (gravel, small stones, waste metal, wood, and plastics) as well as to biotic substrates, e.g., cyanobacterial mats. Microbial biofilms and mats can be also constructed on different biotic and abiotic substrates through bioengineered approaches for the bioremediation of oil-contaminated habitats. Evidences of the bioremediation potential of cyanobacterial mats have been corroborated from the degradation of hydrocarbons emerged of the oil spills from many countries. Cyanobacterial mats and derivative bacteria were able to grow and to degrade most of the oil hydrocarbons present in the oily spills of the coasts of Kuwait. Analysis performed on the microorganisms associated with the degradation of 1% black oil shown that were characterized as belonging to biofilms of Alcanotrophic bacteria associated with

specific microalgae and cyanobacteria (Safonova et al. 2004). Black oil has been reported as effective to enhance the growth of several strains of microalgae and cyanobacteria without any apparent toxic effect, as in Nostoc sp., and Phormidium sp., eukaryotic microalgae (genera Stichococcus sp.), Chlorella sp., and Scenedesmus quadricauda, which in most of the cases were associated with bacterial populations which exerted a protective role to the algae from toxic effect to the oil and contributed to in the biodegradation processes.

In microbial mats, the associated microalgal such as cyanobacteria Oscillatoria, and other cyanobacteria are able to degrade hydrocarbons in oil, although this ability has been explained to its co-association to aerobic bacteria mainly facultative heterotrophs (Safonova et al. 1999). The presence of microbial mats of cyanobacteria or microalgae in lagoons, estuaries, or protected coastal bays and beaches has defined them as natural occurring immobilized microbial systems (Radwan et al. 2002). In aquatic-marine environments, efficient biodegradation and bioremediation of oil hydrocarbons rely its performance in the presence of oxygen which is considered as one of the limiting factors for microbial biodegradative processes of hydrocarbons. It is generally accepted that constructed microbial mats are more affordable to perform bioremediation of C24–C30 n-alkanes or carbazole than those with low molecular weight hydrocarbons or than isoprenoids (chain length lower than C16 or C18) (de Oteyza et al. 2006).

In principle, part of the required oxygen for the biodegradation of aliphatic and aromatic compounds is provided by algal photosynthesis of the microbial mats. Experiments of oil biodegradation were undertaken in a rotating biological contactor, in which the combined action of mat-forming consortia of microorganisms constituted of Phormidium, Oscillatoria and Chroococcus, and the oil-degrading bacterium, *Burkholderia cepacia*, shown a successful removal of total petroleum hydrocarbon.

Other groups of petroleum hydrocarbons are polyaromatic hydrocarbons (PAHs) that are an important part of combustion residues in hydrocarbon compounds. Actually, microorganisms can biodegrade PAHs of only one, two, or three rings. The degradative potential of naturally occurring cyanobacterial mats contributes to hydrocarbon degradation and is in fact mediated though photosynthetic biosurfactants produced by microalgae, cyanobacteria, and bacteria itself (de Oteyza et al. 2006; Rosales and Paniagua Michel 2014; Paniagua-Michel and Rosales 2015).

7 Aquaculture: Biofilms and Mats

Aquaculture is a major method for producing protein, mainly from algae, craceans, molluscs, and fishes (Bellester et al. 2007; Sharma et al. 2011). Despite the successful development of aquaculture industry, unfortunately, is accompanied by negative impacts produced mainly from inefficient food intake, animal feces as well

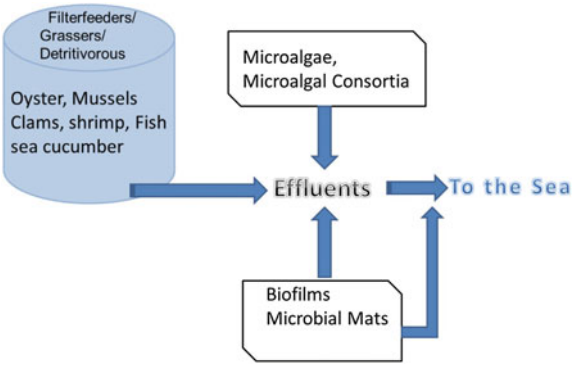

Fig. 4 Integrated aquaculture system for commercially important species involving microalgae, microalgae consortia, biofilms, or mats

as uneaten feed (Gimenez-Casalduero 2000; Lezama and Paniagua Michel 2010). Because the main objective of this technology is the production of live food for human consumption, the preservation of the water quality, and the sustainable environmental conditions should be a priority. Among the alternative solutions to these troubles, the development of integrated aquaculture systems seems to be a practical solution because may utilize environmentally safe and ecological processes on the aquacultural by-products to obtain value-added products, which reduces pollution and increases the profit (Fig. 4). The use of polymeric carriers for the construction of microbial biofilms-mats has been developed in order to accelerate shrimp Litopenaeus vannamei postlarvae production, because microbial community of autotrophic and heterotrophic components of the mats contribute to the enhancement of the nursery conditions, and increase water quality of hatcheries and sustainable protein-food production, as well as refuge and protection of cannibalism (Thompson et al. 2002; Moss and Moss 2004; Lezama Cervantes and Paniagua Michel 2010; Lezama Cervantes et al. 2010). Other attributes of this technology are their ability to decrease water uses and exchange in aquaculture systems, which leads to low-cost production systems.

Advances in the identification and characterization of microbial components of biofilms and mats in shrimp production systems have identified mainly the following taxa, but although species composition may be susceptible to change, the structural taxonomic composition is more consistent. Hence, on top of mats, diatoms and filamentous cyanobacteria are the main taxa, while eukaryotic microalgae, nitrifying bacteria, protozoa, and ciliates are common on lower layers and have been corroborated as being part of the diet of Penaeid shrimps in natural environments (Nunes et al. 1997; Lezama Cervantes et al. 2010).

Actually, progress on the study of that subjected are orientated toward the engineering and improvement of the biofilm composition and management, mainly its content of nutrients and organic–inorganic supplementation on a combination of natural with artificial diets, such as probiotics and functional bioremediation for

aquacultured species. Some authors have decided to use the term biofloc for addressing to similar structures of the microbial biofilm and mats. Originally the term biofloc was derivate from the detached crusts of the biofilms. It is suggested that the use of the term biofloc should be accompanied by terms indicating their uses and applications, viz, aquaculture bioflocs. sewage bioflocs, etc.

One of the remarkable differences between microbial mats and bioflocs is the fact that bioflocs do not keep clear water in the production systems because of its high concentration of total suspended solids, which increases BOD of the culture water and demands of robust aeration systems. contrary to the effect of constructed and natural biofilms and mats that in most of the cases keeps high rates of water filtration leading to "clean water" conditions and low BOD because of the copious production of oxygen by their photosynthetic assemblages in ponds as well as in treatment systems. In recirculating aquaculture systems, the main properties that enables microbial mats to adapt and resist to large variations in dissolved oxygen and pH while removing different organic and inorganic pollutants are associated with the mixed autotrophic and heterotrophic communities from the substrata (Lezama Cervantes and Paniagua Michel 2010; Crab et al. 2012). However, short-cut nitrification–denitrification and anaerobic ammonium oxidation (**anammox**) could benefit production in recirculating aquaculture systems with lower energy demand (Kumar et al. 2010). An update and comprehensive review on the uses of microbial mats in environmental and aquaculture applications have been published by Bender and Phillips (2004) (Fig. 5).

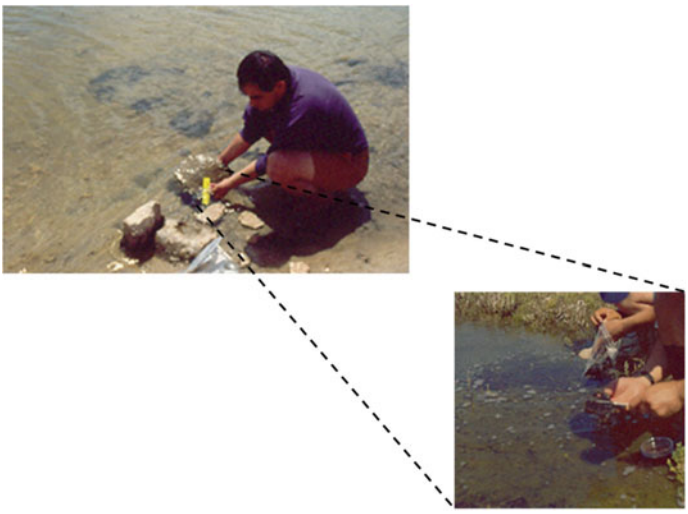

Fig. 5 Sampling natural microbial mats from Guerrero Negro BCS Mexico

8 Engineering Microbial Consortia Forming Biofilms and Mats: Progresses and Expectative

During the last decade, different interacting microbial populations within biofilms and microbial mats and its application in biodegradation and bioremediation processes have led to new developments mainly in aspects of microbial consortia engineering (Fig. 6). In consortia, the different species of microorganisms contribute with their respective arsenal of capabilities to faces and overcome environmental fluctuations as well as other functions that individual microbial populations cannot (Brenner et al. 2008).

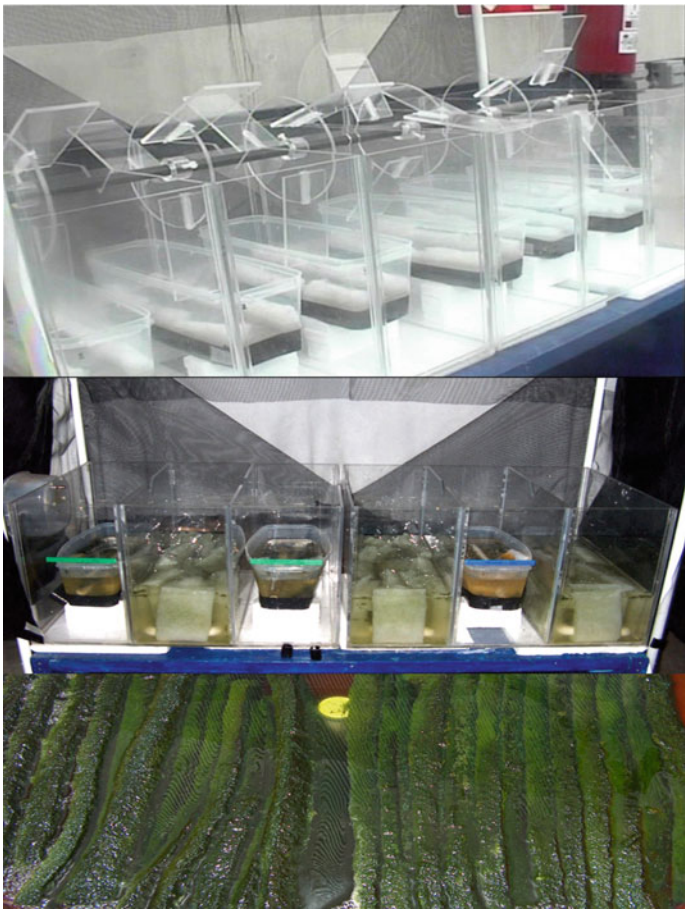

Fig. 6 Procedure for the construction of artificial microbial mats. Top of the figure shows bioreactors with inoculated support containing microbial mats with paddle wheels. Lower figure shows fully developed microbial mats

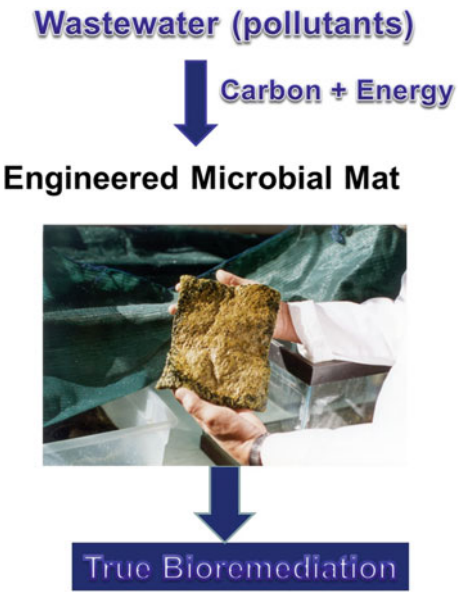

Fig. 7 Engineered microbial mats for multiple uses in bioremediation of wastewater effluents and in aquaculture effluents

During the last decade, different interacting microbial populations within biofilms and microbial mats and its application in biodegradation and bioremediation processes have led to new developments mainly in aspects of microbial consortia engineering (Fig. 6). In consortia, the different species of microorganisms contribute with their respective arsenal of capabilities to faces and overcome environmental fluctuations as well as other functions that individual microbial populations cannot (Brenner et al. 2008). Successful applications of these engineered systems have been reported in the removal of complex and mixed pollutants (Fig. 7), such as are presented in effluents and environments (municipal wastewaters, hydrocarbons, heavy metals, as well as aquaculture effluents). These constructed microbial mats can be immobilized on natural or artificial carriers, most of the times of polymeric, natural or artificial substances. For instance, Paniagua-Michel and Garcia (2003). Zamora et al. (2007) using low-density polyester achieved successful bioremediation of wastewater effluents from municipal treatment plants as well as from shrimp aquaculture effluents (removal efficiencies for NH_4-N, and total NO_3-N higher than 97 and 95% respectively). The structural taxa conformation of the constructed microbial mats was according to the origin of the treated wastewater. In most of the cases, the dominant species were represented by filamentous forms of cyanobacteria (Microcoleus chthonoplastes, Spirulina sp., and Oscillatoria sp.), green algae (Chlorella sp. And Dunaliella sp.), diatoms (Nitzschia sp., and Navicula sp.) as well as representatives of the β- and d —proteobacteria class, mainly nitrifyers, and denitrifyers. In Engineered systems, the algal photosynthetic components of the microbial consortia assures the effective self-oxygenation for the basic processes of bioremediation of most of the pollutants

from wastewater treatment effluents (Munoz and Guieysse 2006). This issue is considered one of the expensive operational costs from conventional and engineering approaches. Among the main capabilities of strains in consortia, the following have been mentioned as important to be considered (Brenner et al. 2008): (a) ability to regulate long-term homeostasis, (b) functionality of consortia despite horizontal gene transfer, and (c) incorporation of stable changes into the genomes of microbial members. Recently, functional genomics and combinatorial biochemical approaches, as well as metabolic engineering, have been complemented by large genomic sequencing programs (Zhou et al. 2004) to develop initiatives toward engineering approaches for the implementation of photoautotrophic and photoheterotrophic algal-bacterial consortia in industrial applications of biofilms and mats. These engineered microorganisms forming biofilms and mats can achieve efficient pollutant degradation ensuring environmental quality and preservation of environment sensible as well as biodiversity-rich habitats. However, still there are challenges needed to overcome in order to progress toward sustainable systems based on engineered microbial biofilms and mats, viz. the selection of microorganisms able to switch-on particular genes involved in overproduction of functional molecules for specific biomitigation purposes and self-control metabolites. Simultaneously, modern molecular advances and omics are contributing to the knowledge of the genetic basis and structure of microbial communities of biofilms and mats in the contaminant biodegradation and bioremediation of wastewater effluents.

Recent developments and modern technological applications and diagnosis, such as atomic force and confocal scanning laser microscopy as well as bioprobes and biomarkers applied to the study of biofilms and microbial mats, have contributed to new discoveries in these systems. For instance, studies in microorganisms from microbial biofilms and mats species have expressed functional genes associated to the biodegradation of wastewater pollutants, but not in free free-swimming species. That is why, aspect related to predators resistance, gene transfer, and exchange, as well as growth, of species are in most of the dynamic assessment models of plankton quite different to the response in biofilms and microbial mats. This is because environmental fluctuations follow a different and diverse metabolic pathway within the mat, mainly in aspects related to light, oxygen and carbon source (Subashchandrabose et al. 2011). Also, interrelationships among members of consortia, such as pollutant mitigation, competition for resources and cooperation among the different population of microorganisms play important roles for successful engineering initiatives of microbial consortia aiming to bioremediate wastewater. Advances related to the metabolic engineering of microbial consortia, mainly in aspects of gene cloning in single organisms of biofilms have shown a promissory feasibility in the expression of genes dependents of the regulatory and metabolic network (Brenner et al. 2008; Subashchandrabose et al. 2011). These findings and omics developments such as metabolic and genomic profiling are scientific-technological tools that should enhance the advances in consortium engineering of phototrophic and photoheterotrophic biofilms and microbial mats for the treatment and bioremediation of wastewater effluents.

9 Conclusion

Modern methods of bioremediation using biofilms and microbial mats are actually considered as a pragmatic, cost-effective, and sustainable treatment option for highly sensitive and fragile marine environments as well as in bioprocesses and production systems, viz, biotechnology, aquaculture, and fisheries. Important roles of biofilms as in their subsidiary matrix of extracellular polymeric substances have led to new applications such as the production of biosurfactants mediated oil hydrocarbons and heavy metals bioremediation, as well as on important processes and interactions (quorum sensing and gene exchange) within the biofilms and microbial mats. The uniqueness of many of these characteristics and particularities of biofilms, and mats have led to innovative strategies benefiting bioremediation and environmentally safe bioprocesses. New and innovative applications based on biofilms and microbial mats, as well as omics and policies and regulations associated with their use, will expand future developments of this biotechnology for biotreatments and remediation mainly in fragile and sensitive environments.

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Algae as Source of Food and Nutraceuticals

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Abstract Increasing knowledge on nutrition and plant biotechnology has changed the concepts of agriculture, food, and its impact on health. The numerous scientific research and publications have shown that algae provide health benefits to the people. Seaweeds, due to the beneficial biochemical composition, have a great potential to be used as components of food aimed at increasing its nutritional value. Algal cells possess a wide diversity of biologically active metabolites, e.g., proteins, vitamins, minerals, polysaccharides, amino acids, fatty acids, lipids and could be used in the development of pharmaceuticals and also essential compounds for human nutrition. They could be used as an antioxidant, anti-inflammatory, anti-coagulant, antimicrobial, antibacterial, antiviral, antifungal, anticancer, antiobesity, antidiabetic, hypercholesterolemic, and antihypertensive nutraceuticals. The nutritional algae value differs from various species, geographic areas, seasons, and water conditions are taken into account. Currently, the algal products have become familiar to people and the prevention of disease is better understood. The number of healthcare pharmaceuticals from algae is still in the development stage and new functions are being investigated.

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B.N. Tripathi and D. Kumar (eds.), *Prospects and Challenges in Algal Biotechnology*, DOI 10.1007/978-981-10-1950-0_10

1 Introduction

Algae have been consumed by humans since ancient times, mainly in Eastern Asia. Seaweeds have been used for the preparation of traditional Japanese sushi and the Korean food gimbap. Algae were also known in Wales as the main component of traditional Welsh laverbread. Increasing consumption of algae attracted scientific interest and a number of publications describing health benefits of these organisms were published (Cassolato et al. 2008; Hiqashi-Okaj et al. 1999). There is a trend for manufacture and development of functional food. Due to the beneficial biochemical composition, algae have a great potential to be used as components of food aimed at increasing its nutritional value (Draaisma et al. 2013; Holdt et al. 2011; Plaza et al. 2010). Micro- and macroalgae are being currently investigated as a potential source of nutraceuticals. The term "nutraceuticals" was defined by the Foundation for Innovation in Medicine (New York, US) in 1989 as a substance constituting food or a part of the food and providing medical benefits (Andlauer and Fürst 2002; Barrow and Shahidi 2007).

2 Algae as Source of Food

The use of algae—more precisely seaweeds—in human nutrition is a traditional practice (Bradford and Bradford 1996; Chapman and Chapman 1980), common in Indian and Pacific countries where algal biomass represents a resource of economical importance (Caliceti et al. 2002). Evaluation of direct seaweed consumption within Japanese population showed average daily intake of 3.3 g, by dry mass (Darcy-Vrillón 1993). Although in Europe, seaweeds are usually considered as utilization-requiring waste rather than a valuable material, hence the algae collection is generally not significant, algal products in the regular diet of Irishmen, Bretons, and Welshmen have been noted (Mouritsen 2013).

Besies harvesting from natural habitat, seaweeds, in the regions of their consumption, are cultivated by aquafarming, particularly in Asia (China, Japan, Korea, and the Philippines) and South America (Chile) (Redmond et al. 2014). Among approximately 250 species of commercially utilized macroalgae, 60% are favorably used as human food (Kumar and Kaladharan 2007). In aquaculture, kelps (*Saccharina japonica* and *Undaria pinnatifida*), tropical red algae (*Kappaphycus* and *Eucheuma* species), nori (*Porphyra* and *Pyropia* species), and agarophytes (*Gracilaria* species) are most commonly cultivated. Worldwide, annual seaweed production provides about 19 million tons of biomass appraised for US $5.65 billion (FAO 2012).

The nutritional algae value differs from various species, geographic areas, seasons, and water conditions are taken into account (Jensen 1993). In general, seaweeds are considered as low-calory food, yet rich in essential fatty acids, vitamins and derivatives, and mineral compounds. High protein and carbohydrate contents in

algal biomass have been proved, as well (Kumar and Kaladharan 2007; Lahaye 1991).

Chapman and Chapman (1980) showed the capability of algae at 100 g dose of fresh mass to provide two-thirds of vitamin C daily requirement and cover the need for vitamins A, B2, and B12. Besides, seaweed-derived vitamin B1, tocopherols, and β–carotene were also reported (Rupérez 2002).

Since algal polysaccharides were verified not to undergo complete digestion in human gastrointestinal tract, they might be applied as dietary fiber—mainly soluble, characteristic of which differs from plant-derived compounds (Kumar and Kaladharan 2007; Urbano and Goñi 2002).

Total mineral composition of algae is known to surpass—up to 40%, by dry mass, terrestrial plant—and animal-based products recorded (Rao et al. 2007); however, along with essential elements-including: Co, Cr, Mo, Ni, Se, V, Mg, Ca, Fe and I (Rupérez 2002), toxic elements—such as: As, Pb or Cd, are also taken up by algae (MacArtain et al. 2007; Ródenas de la Rocha et al. 2009). In the marine environment, mineral bioaccumulation in seaweed tissues—depending on the concentration and bioavailability, might reach several thousand times higher levels compared to surrounding seawater (Dawczynski et al. 2007; Sanchez-Rodriguez et al. 2001).

Pina and co-workes (2014) showed different culinary treatments—such as hydration, boiling and steaming, to affect the valuable compound composition of *Chondrus crispus*. Each examined treatment significantly increased the content of β-carotene (a modified method of Feraces-Casais et al. 2012), while boiling and steaming decreased concentration of phycoerythrin (method of Lage-Yusty et al. 2013) in algae samples.

At the same time, water cooking of *Undaria pinnatifida*, *Laminaria ochroleuca* and *Porphyra umbilicales*, enhanced the release of elements—including chromium and arsenic, into the cooking water, whereas did not change the content of zinc (*L. ochroleuca* and *P. umbilicales*), nickel (*L. ochroleuca*), iron and cobalt (García--Sartal et al. 2013).

3 Algae as Source of Nutraceuticals

The numerous scientific research and publications have shown that phytochemicals in foods and in isolated form provide health benefits to the people (Dillard and German 2000; Espín et al. 2007). The health foods are defined as nutraceuticals and they are introduced according to Nutrition Labeling and Education Act of 1990 and the Dietary Supplement Health and Education Action of 1994 (Dillard and German; 2000) and then the definition of nutraceuticals has broadened with, e.g., vitamins, minerals, herbs, amino acids and dietary substance (Gupta et al. 2010). The term "nutraceuticals" has been introduced by Stephen DeFelice in 1989 through the conjunction of words "nutrition" and "pharmaceutical" (Gupta et al. 2010; Kalra 2003; Vidanarachchi et al. 2012). The nutraceuticals are defined as a food or part of

a food which provides medical or human health benefits including prevention and/or treatment of diseases (i.e., heart disease, cancer, Parkinson's disease, etc.) (Espín et al. 2007; Gupta et al. 2010; Kalra 2003). Nowadays, the consumption of nutraceuticals to improve health, and to prevent and treat diseases receives a greater attention from the global food industry (Vidanarachchi et al. 2012; Zhao 2007). These products are commodities derived from foods but are used in medicine in the form of pills, capsules or liquids (Espín et al. 2007). In the marketplace, there are over 470 nutraceutical and functional food products with proven health benefits (Espín et al. 2007). The global market size for nutraceuticals is 30–60 billion dollars, mainly in the United States, Japan, and Europe. It is estimated that potential short-term growth market demand is over 197 billion dollars. Nutraceuticals contain active ingredients such as polysaccharides, peptides, phytochemicals, vitamins, and fatty acids (FAs). These compounds could be obtained by natural or chemical extraction and biotechnological synthesis (Vidanarachchi et al. 2012). The classification of nutraceuticals is presented in Fig. 1. (Gupta et al. 2010).

In view of still increasing demand for nutraceuticals, organisms with the possibility to rapidly produce nutritional compounds are expected. Among marine organisms, marine algae are rich sources of bioactive compounds (Young-Xin et al. 2011), which are present in nutraceuticals. Currently, the importance of algae as a source of novel bioactive substances is growing rapidly (Miyashita et al. 2011; Young-Xin et al. 2011) and the marine products have become familiar to people and the prevention of disease is better understood (Hu et al. 2016; Miyashita et al. 2011). Among the algae that can be successfully used for the production of nutraceuticals these seaweeds *Nostoc*, *Botryococcus*, *Anabaena*, *Chlamydomonas*, *Scenedesmus*, *Synechococcus*, *Parietochloris,* *Porphyridium,* etc., can be

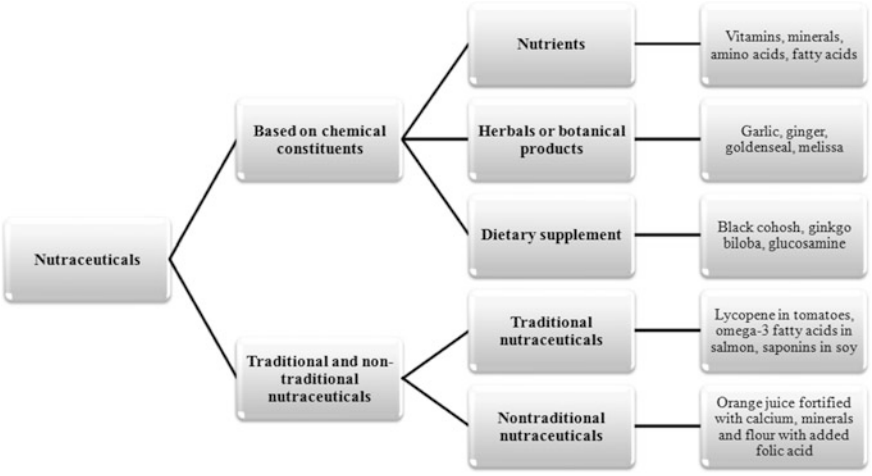

Fig. 1 The classification of nutraceuticals (Gupta et al. 2010)

mentioned. Algae possess a wide diversity of biologically active metabolites (e.g., proteins, vitamins, minerals, polysaccharides, amino acids, Omega 6, Omega 3 fatty acids, lipids) (Miyashita et al. 2011), as an aid to protect from other organisms. This property could be used for the development of pharmaceuticals and also essential compounds for human nutrition (Dai-Hung and Se-Kwon 2013; Thanh-Sang et al. 2012a, b; Young-Xin et al. 2011). In this work, some of the algal nutraceuticals are described.

3.1 Antioxidant Nutraceuticals

Reactive oxygen species (ROS) and free radicals are effectively eliminated by the healthy cells by the enzyme-mediated system (e.g., peroxidase, glutathione) or non-enzymatic factors (e.g., ascorbic acid, protein). When the balance between the pro-oxidant formation and antioxidant capacity is not supported, the oxidative damage will occur and cause pathophysiological effects (Sheih et al. 2009). For example, lipid peroxidation may cause such effects (Matsukawa et al. 1997). Reactive oxygen species (ROS), which can attack key biological molecules such as DNA, RNA, lipids, proteins, contribute to arise of a wide range of common diseases (e.g., cardiovascular disease, inflammatory conditions, and neurodegenerative disease) and age-related degenerative conditions (Kuda et al. 2005; Sheih et al. 2009; Zhang et al. 2010). Currently, to retard the oxidation process, the synthetic antioxidants (e.g., butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate) are used (Sheih et al. 2009), but they must be under strict regulation due to potential toxic effects (Kuda et al. 2005; Sheih et al. 2009). For this reason, the development of alternative natural antioxidants is of great importance for our health (Hu et al. 2001). A number of studies have pointed out the antioxygenic activities of seaweeds (Tutour et al. 1998). These algae live in harsh conditions and are exposed to a combination of solar radiation, high oxygen concentrations (Matsukawa et al. 1997) and high temperature, which normally leads to the formation of strong oxidizing agents. Algae have developed a number of defense mechanisms against ultraviolet radiation and excessive production of free radicals through the accumulation of antioxidant substances (López et al. 2011; Matsukawa et al. 1997). Many reports confirm that marine algae constitute the vast array of reactive antioxidant ingredients (e.g., L-ascorbic acid, glutathione (GSH), polyphenols, phylopheophytin, fucoxantinein) (Demirel et al. 2009; Kuda et al. 2005; López et al. 2011; Wang et al. 2010), as well as secondary metabolites, including carotenoids (α- and β-carotene, fucoxanthin, astaxanthin), catechins (e.g., catechin, epigallocatechin, epigallocatechin), gallate, phlorotannins (e.g., phloroglucinol), eckol and tocopherols (α-, χ-, δ-tocopherols) (Demirel et al. 2009; Matsukawa et al. 1997) which are believed to help prevent aging and many degenerative diseases such as cardiovascular diseases and cancers (López et al. 2011).

Wang et al. (2010) investigated the effect of extraction of polyphenols and other antioxidant ingredients from *Palmaria palmata* and antioxidant activities of the hydrolysates. Results showed that all tested proteases had a significant enhancing effect on the extraction of polyphenols and other active components compared to carbohydrates and cold water extraction (control). The Umamizyme extract had the highest total phenolic content and consequently exhibited the strongest scavenging capacity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and peroxyl radicals. The data from this study suggest the potential of protease treatment to improve value-added utilization of dulse extracts as antioxidants in functional foods and nutraceuticals.

The antioxidant activity of sulfated polysaccharides, extracted from five algae: *Laminaria japonica* (brown algae), *Porphyra haitanensis* (red algae) and *Ulva pertusa*, *Enteromorpha linza* and (green algae), were examined by Zhang et al. (2010). The antioxidant activities of all the samples were investigated including scavenging effects of superoxide and hydroxyl radicals. Authors found that all samples possessed antioxidant activities in certain assays.

The experimental data presented in López et al. (2011) work showed that water, water/methanol (1/1), methanol and ethanol crude extracts from *Stypocaulon scoparium* have a significant association between the antioxidant properties and total phenolic content. The aqueous extract showed both, the highest antioxidant activity and highest phenolic contents. In Table 1, other algae species with antioxidant activity are presented.

3.2 Antimicrobial Nutraceuticals

Investigators have documented increasing resistance of clinically important bacteria (e.g., *Staphylococcus aureus*) to known antibiotics (e.g., penicillium). The use of new compounds, not based on existing synthetic agents, is one of the ways of preventing antibiotic resistance (Eom et al. 2012). Algal antimicrobial compounds can play a significant role as nutraceuticals for preventing the disease risk, also can

Table 1 Different antioxidant algal compounds

Algae	Active compound	Reference
Scytosiphon lomentaria, Papenfussiella kuromo, Nemacystus decipiens	Catechin	Kuda et al. (2005)
Chlorella vulgaris	Protein	Sheih et al. (2009)
Laminaria digitata, *Himanthalia elongata*, *Fucus vesiculosus*, *Fucus serratus, Ascophyllum nodosum*	Fucoxanthin	Tutour et al. (1998)
Colpomenia sinuosa, *Dictyota dichotoma, Dictyota dichotoma* var. *implexa, Petalonia fascia, Scytosiphon lomentaria*	Phenols	Demirel et al. (2009)

improve the shelf life of food and prevent the effects of food-borne pathogens (Sasidharan et al. 2010; Taskin et al. 2010; Vidanarachchi et al. 2012). To screen algae extracts for antimicrobial activity the diffusion methods are widely used. This approach is simple, practical and recommended by the National Committee for Clinical Laboratory Standards (Abu-Ghannam and Rajauria 2013). Algal secondary metabolites such as fatty acids, halogenated compounds, alcohols, hydroquinones, aldehydes, ketones, carbonyls, and phlorotannins are regarded as compounds which can be used as an effective therapy against drug-resistant bacteria (Abu-Ghannam and Rajauria 2013). The majority of compounds responsible for antimicrobial activity are polysaccharides and phenolics. The antimicrobial mechanism could be stasis (growth inhibition of microorganism) or cidal (destruction of microorganisms) (Davidson and Naidu 2000). The isolated and characterized phlorotannins (e.g., phloroglucinol, eckol, fucofuroeckol-A, phlorofucofuroeckol-A, and dieckol) show an antimicrobial effect against food-borne pathogenic bacteria, antibiotic resistance bacteria, and human *tinea pedis* fungus (Eom et al. 2012). Carotenoids also exhibit antimicrobial activity but their mechanism of action is still not clear (Cucco et al. 2007). Due to algal antimicrobials could be successfully used as nutraceuticals in the food industry (Vidanarachchi et al. 2012). In Table 2, other algae species with antimicrobial activity are presented.

3.3 Antiallergic Nutraceuticals

Allergic diseases are one of the main public health problems, which affect about one-third of the population in the developed world (Le et al. 2009; Thanh-Sang et al. 2012a, b). Essentially, allergy is an effect of hyperbolical reaction of an organism to environmental substances (e.g., dust mites, animal dander, foods,

Table 2 Different antimicrobial algal compounds

Algae	Microorganism	Reference
Asparagopsis taxiformis, Cymopolia barbata	Gram-positive bacteria	González del Val et al. (2001)
Asparagopsis taxiformis, Osmundea hybrida	Mycobacteria	González del Val et al. (2001)
Gracilaria changii	*Pseudomonas aeruginosa*	Sasidharan et al. (2010)
Cystoseira compressa, Cystoseira crinita, Cystoseira sedoides, Gelidium latifolium, Dictyopteris membranaceae and *Halurus equisetifolius*	*Staphylococus aureus, Echerichia coli, Staphylococcus epidermis, Micrococcus luteus*	Mhadhebi et al. (2012)
Scytosiphon lomentaria	*Salmonella typhimurium*	Taskin et al. (2010)

pollen, and chemical agents) (Kim 2011; Li et al. 2008; Thanh-Sang et al. 2012a, b), affecting immunological or chemical activation of mast cells which leads to release of inflammatory mediators such as: histamine, proteases, proteoglycans, eicosanoids and chemotactic, proinflammatory cytokines such as: interleukin (IL-6, IL-4, IL-13) and tumor necrosis factor (TNF)-a (Le et al. 2009). Histamine is the best-characterized potent vasoactive mediator and marker of degranulation (Li et al. 2008). A great source for the advancement of new generation of antiallergic drugs, which are more effective and show less side effects (Thanh-Sang et al. 2012a, b), could be marine algae, which show high inhibitory effect on the degranulation (Le et al. 2009; Thanh-Sang et al. 2012a, b), attenuating the release of histamine, β-hexosaminidase and cytokines (Thanh-Sang et al. 2012a, b). Among the algal extracts, which have been found to be efficient for antiallergic therapeutics (inhibit more than 50% of β-hexosaminidase), e.g., extracts of *Petalonia binghamiae*, *Chrysymenia wrightii*, *Scytosiphon lomentaria*, *Ecklonia cava*, *Undaria pinnatifida*, *Codium fragile*, *Porphyra dentate,* and *Ulva japonica* (Thanh-Sang et al. 2012a, b). Experimental research on model animals such as mice or rats confirmed that components, e.g., polyphenols, oligosaccharides, lipids, some unsaturated fatty acids have antiallergic activity (Sugiura et al. 2008). The potential antiallergic compounds derived from marine algae may be included: fucoidan, alginate (Sugiura et al. 2008), phlorotannins, polysaccharides, carotenoids, polyunsaturated fatty acids, and phycocyanins (Vo et al. 2012). Some antiallergic investigations for components of algae have already been reported.

Samee et al. (2009) showed that ethanol extracts of brown seaweeds: *Sargassum tenerrimum* (ST), *Sargassum cervicorne* (SC), *Sargassum graminifolium turn* (SG), *Sargassum thunbergii* (STH), and *Laminaria japonica* (LJ)-contained phlorotannin (an inhibitor against hyaluronidase) in amount 5.06%, 3.30%, 1.71%, 0.74%, and 0.97%, respectively. The 50% inhibitory concentrations (IC50) of SC and ST were 109.5 and 21 μg/ml, respectively, lower than those of SG, STH, and LJ (134, 269, and 148 μg/ml, respectively). The IC50 of ST extract was found similar to that of a natural inhibitor of hyaluronidase, catechin, (21 vs. 20 μg/ml) and lower than that of an antiallergic drug, disodium cromoglycate, (21 vs. 39 μg/ml). Authors suggest that ST could be used as a potent, natural inhibitor of hyaluronidase.

Sugiura et al. (2007) also reported inhibitory activity upon β-hexosaminidase, release from the rat basophilic leukemia-2H3 cells, of extract from *Eisenia arborea*. Most of the phlorotannins present in that brown alga exhibited activities similar to or greater than the typical inhibitor (epigallocatechin gallate), e.g., phlorofucofuroeckol-B showed the greatest activity among the tested phlorotannins at 2.8 times greater than epigallocatechin gallate.

Sugiura et al. (2015) determined the antiallergic effects of *Ecklonia kurome.* To determine the inhibitory effects on inflammation, degranulation in inflammatory lymphocytes and enzymatic activities involved in allergic actions the ethyl acetate fraction from *E. kurome* (EEK) extract was compared to the ethyl acetate fraction from an *Eisenia arborea* (EEA) extract and epigallocatechin gallate (EGCG). EEK inhibited mouse ear edema by inflammatory inducers (arachidonic acid, 12-Otetradecanoylphorbol-13-acetate and oxazolone) through both topical and oral

administration. EEK also inhibited degranulation in rat basophilic leukemia cells and enzymatic activities (cyclooxygenase-2, soybean lipoxygenase, and phospholipase A2). The obtained results for EEK were generally comparable with those for EEA and EGCG.

According to Li et al. (2008), the antiallergic activities of two bioactive phloroglucinol derivatives (fucodiphloroethol G and phlorofucofuroeckol-A) isolated from *Ecklonia cava* were assessed on human basophilic leukemia (KU812). Both tested compounds (in the crude methanol extracts) exhibited a significant inhibitory activity against histamine release. Bae et al. (2013) have clearly demonstrated that extract of *Chlorella vulgaris* (CVE) acted as an antiallergic dietary agent by suppressing histamine release in oral administration (2 g/kg CVE) in ovalbumin-sensitive mice. In Table 3, other algae species with antiallergic activity are presented.

3.4 Antiobese Nutraceuticals

Decreased physical activity and unhealthy diet are the main reasons of metabolic and inflammatory diseases, such as adiposity (Gammone and D'Orazio 2015).

Table 3 Different antiallergic algal nutraceuticals

Algae	Active compound	Inhibitory effect/Allergy	Reference
Sargassum tennerimu	Phlorotan	Inhibitor of hyaluronidase	Samee et al. (2009)
Eckolonia cava	Phloroglucinol derivatives, fucodiphloroethol G and phlorofucofuroeckol	Human basophilic leukemia (KU812) and rat basophilic leukemia (RBL-2H3) cell lines	Li et al. (2008)
Laurencia undulata	Polyphenols	OVA-induced murine allergic airway reactions	Jung et al. (2009)
Eisenia arbore	Phlorotann, phlorofucofuroeckol-B	Inhibitory effect on histamine release from rat basophile leukemia (RBL)-2H3 cell	Sugiura et al. (2006)
Eisenia arborea	Phlorofucofuroeckol-B	Inhibitory activity upon β-hexosaminidase release from the rat basophilic leukemia-2H3 cells	Sugiura et al. (2007)
Eisenia bicyclis and *Ecklonia kurome*	Phlorotannins	Inhibitor of hyaluronidase	Shibata et al. (2002)
Ecklonia cava	Phlorotannins	Inhibition of histamine release assay on human basophilic leukemia (KU812) and rat basophilic leukemia (RBL-2H3) cultured cell lines	Le et al. (2009)

Obesity is a medical condition which fundamentally results from a persistent imbalance between energy intake and energy expenditure, causing excessive fat accumulation (especially in white adipose tissue) (Jung et al. 2014a; Kang et al. 2012, 2016; Maeda et al. 2009; Vo and Kim 2013). The prevalence of obesity has dramatically risen in the past decades (Hu et al. 2016) since this disorder is a known risk factor for many chronic conditions, primarily heart disease, type 2 diabetes, obstructive sleep apnea, cancer, osteoarthritis, cerebrovascular disease, cholelithiasis, hypertension, hyperlipidemia, pulmonary embolism, stroke, gallbladder disease, gynecological abnormalities, osteoarthritis, psychiatric illness, nonalcoholic fatty liver disease, malignancy, and hyperuricemia (Hu et al. 2016; Jung et al. 2014a; Kang et al. 2012, 2016; Vo and Kim 2013). Obesity is a leading preventable cause of death worldwide (Kang et al. 2012) and annually at least 2.8 million adults die because of the obesity and overweight (Kang et al. 2016). This disease was considered as one of the most serious public health problem of the 21st century (Kang et al. 2012), which costs 2 trillion dollars at a global scale every year (Hu et al. 2016). The World Health Organization informs that more than 1 billion people are overweight (thereabouts 400 million of them are obese) worldwide (Hu et al. 2016). The body mass index (BMI), calculated by dividing body weight in kg by square of height in meters, is the most common standard for estimating a person as obese or overweight (BMI of 30.0 or higher) (Hu et al. 2016). Obese is correlative with the intracellular lipid accumulation, lipolysis and the extent of adipocyte differentiation (Vo and Kim 2013). In case of obesity and related disorders, the knowledge about development and regulation of adipocyte is very important, because it plays a crucial role in energy balance, insulin sensitivity, and immune response by releasing adipokines such as adiponectin, leptin, and resistin (Janovská et al. 2013; Jung et al. 2014b). The suppression of adipogenesis could regulate the amount of adipose tissue mass. Adipokine exerts direct influence on the development of fat cells (Jung et al. 2014a). CCAAT-enhancer-binding proteins (C/EBPs), peroxisome proliferator-activated receptors γ (PPARγ), and sterol regulatory element binding proteins (SREBPs) are involved in the differentiation of adipocytes (Jung et al. 2014b; Kang et al. 2012). For example, PPARγ is highly expressed in adipose tissue, where it plays an essential role in adipogenesis and lipid homeostasis (Jung et al. 2014a). 3T3-L1 cells (mouse embryonic fibroblast pre-adipocytes) have long served as a well-established in vitro assay system for assessing adipogenesis and adipocyte differentiation (Jung et al. 2014a, b). In the prevention of obesity, the dietary intervention, physical activity, pharmacological therapy, and stomach surgery (either alone or in combination) could be used (Hu et al. 2016). Among drugs, there are sibutramine and orlistat, but they have some adverse effects, such as a headache, thirst, insomnia, constipation, and steatorrhea (Kang et al. 2016). Having regard to the most people lifestyle changing is difficult to persist in, also surgical procedures may induce many kinds of side effect, nutritional and dietary factors should be developed as an alternative option of obesity treatment and prevention (Hu et al. 2016). One of the natural remedies to combat life-threatening obesity (Kang et al. 2012; Kang et al. 2016) are marine

algae (Gammone and D'Orazio 2015; Hu et al. 2016). Algal cells possess compounds with confirmed antiobese activity (e.g., polyphenols, phlorotannins (Jung et al. 2014a), lipids, polysaccharides (fucoidan), carotenoids (fucoxanthin)) (Hu et al. 2016). For example, fucoidan has been verified as an inhibitor for fat accumulation, which is mediated by suppressing gene expression of fatty acid binding proteins, acetyl CoA carboxylase, and peroxisome proliferation-activated receptor c (Vo and Kim 2013).

Jung et al. (2014a) isolated five phlorotannins (phloroglucinol, dieckol, eckol, dioxinodehydrockol and phlorofucofuroeckol) from ethyl acetate fraction of *Ecklonia stolonifera* and screened them for abilities to inhibit adipogenesis over a range of concentrations (12.5–100 μM). These phlorotannins significantly reduced (in different extents) the expression levels of several adipocyte marker genes (proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα)). Authors suggest that the molecular weight of a phlorotannin is an important factor affecting its ability to inhibit adipocyte differentiation.

Moreover, Jung et al. (2014b) also evaluated the possibility of the use of sterol, fucosterol, extracted from *Ecklonia stolonifera,* for the inhibition of adipocyte differentiation and lipid formation. The methanolic extract of *E. stolonifera* showed strong anti-adipogenic activity. The MeOH extract was then separately suspended in several solvents. The most active fraction (40.5% of inhibition of intracellular lipid accumulation at nontoxic concentration) was found to the dichloromethane fraction. Positive results were also obtained for the ethyl acetate fraction at the same concentration (30.2%). The n-butanol and water fractions did not show inhibitory activity within the tested concentrations. Fucosterol obtained from the CH_2Cl_2-soluble fraction inhibits expression of PPARc and C/EBPa, resulting in a decrease of lipid accumulation in 3T3-L1 pre-adipocytes, indicating that the potential use of *E. stolonifera* and its bioactive fucosterol as an antiobesity agent.

Kang et al. (2016) showed that ethanol extracts from *Plocamium telfairiae* (PTE) inhibited fat accumulation and adipogenesis by 42.08, 26.41, and 17.86% at the concentrations of 25, 50, and 100 μg/mL, respectively. Oral administration of PTE significantly reduced the body weight, fatty liver, amount of white adipose tissue, and levels of triglycerides and glucose in the tested mice. According to these results, the extracts from *Plocamium telfairiae* could be used as a therapeutic agent for obesity.

Kang et al. (2012) investigated the antiobesity of *Petalonia binghamiae* extract (PBE) in obese mice. The oral administration (150 mg/kg/day for 70 days) of PBE decreased body weight gain, adipose tissue weight, and the serum triglyceride level in mice fed with a high-fat diet. The fucoxanthin obtained from PBE increased the phosphorylation of AMPK-activated protein kinase and acetyl CoA carboxylase with increasing liver kinase B1 phosphorylation in mature 3T3-L1 adipocytes. These results suggest that *Petalonia binghamiae* extract and fucoxanthin promote β-oxidation and reduce lipogenesis. In Table 4 other algae species with antiobese activity are presented.

Table 4 Different antiobese algal nutraceuticals

Algae	Active compound	Model animal	Reference
Undaria pinnatifida	Fucoxanthin	Mice	Woo et al. (2009)
Undaria pinnatifid	Fucoxanthin	KK-Ay obese mouse	Meada et al. (2007)
Ecklonia cava	Phlorotannin	C57BL/6 male mice	Park et al. (2012)
Hematococcus pluvialis	Astaxanthin	Male Swiss albino mice	Arunkumar et al. (2012)

3.5 Antitumor Nutraceuticals

Cancer, the major cause of death, is a group of disorders which is characterized by the uncontrolled proliferation of anaplastic cells which have a tendency to invade surrounding tissues and metastasize to other normal tissues and organs (Senthilkumar and Kim 2014). These debilitating diseases afflicted the entire population worldwide in all generations (Moghadamtousi et al. 2014). On the mutation in the chromosomal DNA of the normal cell, which promotes cancer progression, may influence the external factors (e.g., tobacco (25–30%), alcohol (30–35%), genetic defects 15–20%, radiation (10%), obesity, poor diet, chemicals, and infectious agents (Hussain et al. 2016; Pádua et al. 2015; Senthilkumar and Kim 2014), but also the internal factors (hormones, immune conditions, inherited mutations, and mutations occurring in metabolism) (Pádua et al. 2015; Senthilkumar and Kim 2014). Cancers can be classified on the basis of the type of cells, such as carcinoma, sarcoma, lymphoma, and leukemia, germ cell tumor, and blastoma. Thus, far are known approximately 100 kinds of cancer which affect humans (Hussain et al. 2016) and the breast cancer is considered to be one of the most common cancers and its incidence tends to rise every year (Kazłowska et al. 2013; Pádua et al. 2015; Kim et al. 2015). The side effects, such as bleeding, hair loss, diarrhea, and immunosuppression, associated with currently used chemotherapeutic drugs, and drug-resistance is a frequent problem which has led to search for a development of new anticancer natural products and metabolites which possesses high efficacy against tumor cells without any toxicity on normal cells (Moghadamtousi et al. 2014) which seems to be a crucial strategy to reduce mortality and improve life quality of patients (Pádua et al. 2015). Many studies report that people who consume algae every day are less subjected to cancer (Hussain et al. 2016; Pádua et al. 2015). Hitherto reported that about 15,000 compounds have been discovered from seaweeds, and several antitumor compounds are currently being investigated through clinical trials (Hussain et al. 2016; Schwartsman et al. 2001). The increasing number of in vivo studies and in vitro examinations of crude extracts of brown seaweed, containing, e.g., phloroglucinol, fucoidan, fucoxanthin and phenols, showed a promising anticancer potential (Table 5) (Hussain et al. 2016; Menshova et al. 2015; Moghadamtousi et al. 2014; Pádua et al. 2015).

Table 5 Different anticancer algal nutraceuticals

Algae	Active compound	Type of cancer	Model animal	Reference
Fucus evanescens	Fucoidan	Lewis lung adenocarcinom	C57Bl/6 mice	Alekseyenko et al. (2007)
E. binghamiae	–	HCT-116 colon cancer cells	–	Villarreal-Gómez et al. (2010)
Green sea algae	Dimethylsulfoniopropionate	Ehrlich ascites carcinoma	Bearing mice	Nakajima et al. (2009)
Amphiroa zonata	–	Human leukemic cell lines (L1210)	–	Harada and Kamei (1997)
Gracillaria corticata	–	Human cancer cell lines (MCF-7)	–	Namvar et al. (2014)
Dunaliella bardawil	β-carotene	Neoplastic mammary cells	SHN virgin mice	Fujii et al. (1993)
Monostroma nitidum	Sulfated polysaccharides	Human cancer cell line (AGS)	–	Karnjanapratum and You (2011)
Cladophora glomerata	–	KB human oral Cancer cell lines	–	Laungsuwon and Chulalaksananukul (2013)
Eucheuma cottonii	Polyphenol	Breast cancer	–	Farideh et al. (2012)

Kim et al. (2015) found that phloroglucinol (PG) is a good candidate to target BCSCs and to prevent the disease relapse. This natural phlorotannin component of brown algae suppresses sphere formation, anchorage-independent colony formation, in vivo tumorigenicity, decreased CD44+ cancer cell population as well as expression of CSC regulators (e.g., Sox2, CD44, Oct4, Notch2, β-catenin).

Lakmal et al. (2014) explored the anticancer activity of three species of red algae (*Chondrophycus ceylanicus*, *Gelidiella acerosa*, *Gracilaria corticata*), two species of green algae (*Chaetomorpha crassa*, *Caulerpa racemosa*) and one species of brown algae (*Sargassum cassifolium*) against different cancer cell lines including a human promyelocytic leukemia (HL-60), a human lung carcinoma (A549) and a mouse melanoma (B16F10). A significant cancer cell growth inhibitory effect (IC50 value 30.17 μg/mL) was observed by *C. racemosa* methanol extract against HL-60 cells and it was the highest anticancer effect compared to the other extracts.

Kazłowska et al. (2013) evaluated in vitro and in vivo the protective effect of a sterol fraction from *Porphyra dentata* against breast cancer linked to tumor-induced myeloid derived-suppressor cells (MDSCs). The methanol extract of *P. dentata*, contained a sterol fraction, cholesterol, β-sitosterol, and campesterol, significantly inhibited cell growth and induced apoptosis in 4T1 cancer cells in vitro and increased the survival rate of mice.

4 Conclusions

Nutritional awareness of people caused increased demand for the development of functional food. The number of healthcare pharmaceuticals from algae is still in the development stage and new functions are being investigated. The growing evidence demonstrating the great potential of algae for the manufacture of food and nutraceuticals should give rise to the expansion of pro-health value products in food and pharmaceutical industry.

Acknowledgments This project was financed in the framework of a grant entitled "Innovative technology of seaweed extracts—components of fertilizers, feed and cosmetics" attributed by The National Centre for Research and Development in Poland, project No PBS/1/A1/2/2012.

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Algae as Source of Pharmaceuticals

Agnieszka Dmytryk, Łukasz Tuhy and Katarzyna Chojnacka

Abstract Pharmaceuticals or pharmaceutical products are medicinal drugs—of proven safety, effectiveness and high quality, which are prescribed and intended to rational dosage. In general, most of the pharmacologically active compounds were isolated from microorganisms and plants, drug-resistance and identification of new disease entities have imposed to select both new sources and application areas of drug components. There is a broad range of health disorders—including cancer, allergy, diabetes, neurodegenerative diseases and inflammation, against which algae have been widely used. Medicinal application of algae depends on the biochemical diversity which is affected by a number of factors, including location, season, grazing pressure, salinity, water motion, temperature, light climate, biomass density and nutrient availability. Despite algae variability, main groups of compounds—such as polysaccharides, pigments, terpenoids, alkaloids, polyphenols, peptides and polyunsaturated fatty acids—showing pharmaceutical activity are indicated. Algae constitute an abundant source of bioactive compounds which have a great potential to be used as pharmaceuticals. Currently, the growing interest is put on the application of different algal compounds in the civilization diseases treatment and the market for pharmaceuticals based on compounds of natural origin is growing worldwide. The still untapped reservoir of chemically active compounds and potential in the field of pharmaceuticals imply a requirement of increased screening of algae for healthcare chemicals and the isolation methods development.

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B.N. Tripathi and D. Kumar (eds.), *Prospects and Challenges in Algal Biotechnology*, DOI 10.1007/978-981-10-1950-0_11

1 Introduction

Pharmaceuticals or pharmaceutical products are medicinal drugs—of proven safety, effectiveness and high quality, which are prescribed and intended to rational dosage (www.who.in). In general, most of the pharmacologically active compounds were isolated from microorganisms and plants, drug-resistance and identification of new disease entities have imposed to select both new sources and application areas of drug components (Schwartz et al. 1990).

The beneficial effect of algae-based products on human health has been known since antiquity, their use was, however, limited to folk medicine (Hoppe 1979). Due to the problematic transfer of species from natural habitats to the laboratory-scale cultivation and controversies over the results of algae blooms in aquatic life, the introduction of products to the market was difficult (Garham and Carmichael 1979; Moore 1977; Shimizu 1978). Although the first report on algae medicinal application was 'Materia Medica' by Shên-nung in 2700 B.C. (Hoppe 1979), the systematic investigation of algal active compounds—particularly antibiotics, began after World War II (Borowitzka 1995) as lateral direction of studies on unconventional protein sources for increasing human population. Early research verified algae for pharmaceutical activity mainly by in vitro experiments, subjected to public discussion in the late 1960s (Hoppe 1979). In vivo examination, on the other hand, was elaborated in the 1970s at the Roche Research Institute of Marine Pharmacology (Australia) and involved screening of crude extracts, rather than pure compounds, to isolate and identify components with given properties (Baker 1984). At the same time, in vitro screens were advanced and novel designs—assessing enzyme activity variation or cell line behaviour, enabled to develop efficient and low-cost investigation methods. These approaches are commonly used nowadays (Borowitzka 1995; Patterson et al. 1991; Suffness et al.1989). As greater attention was paid to marine source for medicinal application, microalgal biomolecules became products of interest (Borowitzka 1995; Kellam and Walker 1989; Moore et al. 1988; Patterson et al. 1991). At present, algae-based pharmaceuticals belong to small, yet high-value and mid-sized/value markets being appraised for $25–5800 thousand and $2–25 thousand per tonne, respectively (www.biofuelsdigest.com). According to the report of BCC Research from 2011, the global market of the marine-derived drug is expected to increase at compound annual growth rate of 12.5% reaching $8.6 billion by 2016 (BCC Research 2011).

There is a wide range of health disorders—including diseases of the circulatory and digestive systems, goitre and inflammation (Hoppe 1979), against which algae have been traditionally used. Medicinal application of algae depends on biochemical diversity which is affected by a number of factors, including location, season, grazing pressure, salinity, water motion, temperature, light climate (depth, turbidity and UV exposure), biomass density and nutrient availability (Stengel et al. 2011). The main groups of pharmaceutical compounds and their activities are shown (Fig. 1).

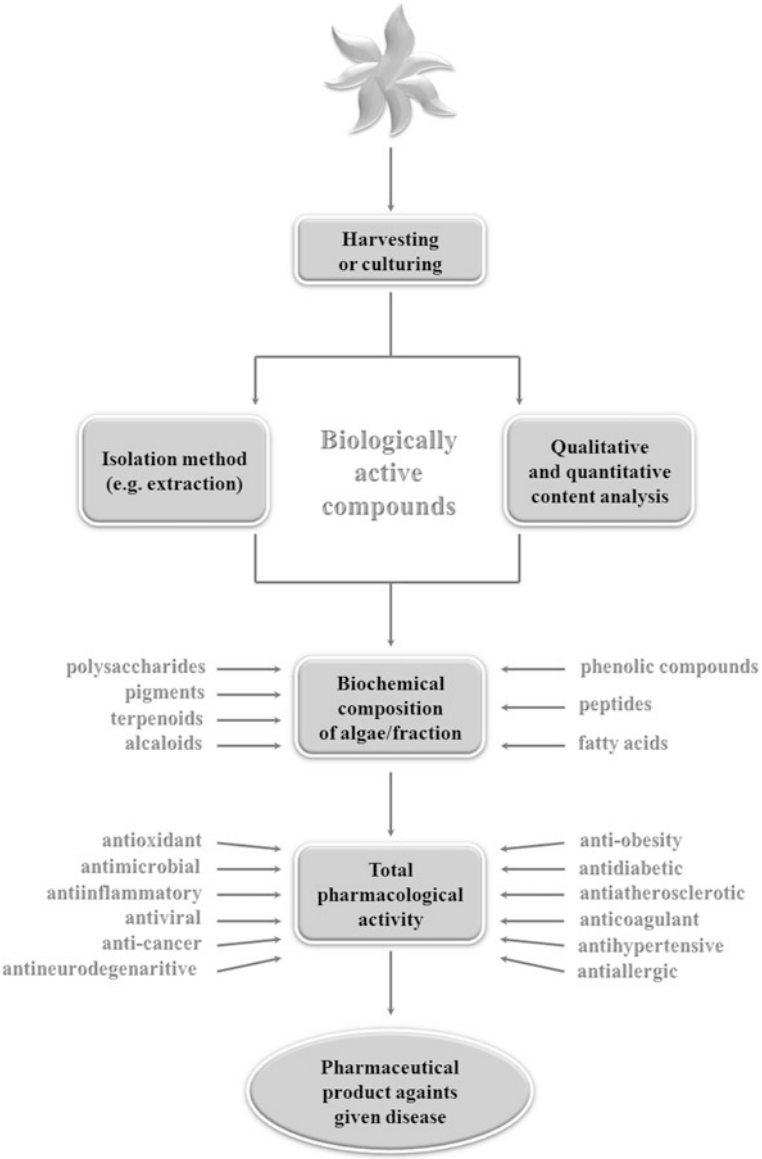

Fig. 1 Scheme of obtaining pharmaceutical products from algal biomass

It is a common practice to evaluate the applicability of selected source, based on total biological activity assessed, and pharmaceutical products comprised of pure components rather than a mixture of given properties. Thus, in the current work, review section was divided into seven subsections, each of which described one group of algae-derived compounds.

2 Compounds with Pharmaceutical Potential

2.1 Polysaccharides

Polysaccharides constitute the most widespread group of chemically active compounds found in algae and demonstrating pharmaceutical properties. Antiinflammatory activity of polysaccharides was shown to be a feature dependent on the type of molecule and its biological source. Polysaccharides are able to bind to the surface of leukocytes and decrease inflammation by interference with the migration of white blood cells (Raposo et al. 2015).

Yang et al. (2008) extracted fucoidans from *Undaria pinnatifida* and observed the anticancer activity of isolated polysaccharides. It was also shown that partial depolymerization of fucoidans in mild conditions significantly improved the anticancer activity of extracted biomolecules (Yang et al. 2008).

The activity of fucoidan from *U. pinnatifida* towards PC-3 prostate cancer cells was examined by Boo et al. (2013). Extracted polysaccharide induced apoptosis and inhibited the growth of tumour cells through the activation of ERK1/2 MAPK and inactivation of p38MAPK pathways (Boo et al. 2013).

Fucoidan obtained from *Cladosiphon novae-caledoniae* enhanced activity of chemotherapeutic agents such as cisplatin, tamoxifen and paclitaxel in breast cancer treatment. Its application induced apoptosis and reduced expression of Bcl-xL and Mcl-1 (Zhang et al. 2013).

In another study, fucoidan led to apoptosis induction in MCF-7 tumour cells by the activation of caspase 8 (Yamasaki et al. 2012).

Fucoidans extracted from *Laminaria cichorioides* and *Fucus evanescens* were examined for their influence on blood coagulation system. Dose-dependent inhibition of thrombin and Xa factor as a result of different sulfation degree of polysaccharides was observed (Drozd et al. 2006).

Fucoidan was also shown to be a factor able to inhibit binding formation between host cell and virus and hence a great antiviral agent towards HSV, RSV and HIV (Smit 2004).

Fucoidan was also recognized to be effective against allergic response. Polysaccharides isolated from *U. pinnatifida* caused the reduced concentration of interleukins in bronchoalveolar lavage fluid and suppressed production of IgE in airway hypersensitivity (Maruyama et al. 2005).

The treatment of mouse thymocytes with different concentrations of laminarin extracted from *Laminaria japonica* led to apoptosis suppression and genes responsible for the production of immune response proteins were activated (Kim et al. 2006a).

Laminarin produced by *Laminaria digitata* was examined for human colon cancer treatment. It was observed that extracted polysaccharide induced apoptosis of HT-29 cells and activated ErbB2 phosphorylation (Park et al. 2013).

Inhibited proliferation and induced apoptosis after the application of laminarin for prostate cancer PC-3 cells was observed in the study conducted by Zou et al. (2010). Also, increased expression of P27kip1 and PTEN was investigated.

Laminarin showed also beneficial effect against RIF-1 tumour cells. In vitro experiments proved its influence on the prevention of tubule formation. Reduced tumour growth as a result of laminarin application was also observed in the in vivo experiments (Hoffman et al. 1996).

Among algal polysaccharides with pharmacological potential, alginate should also be mentioned. Experiments carried out by Asada et al. (1997), alginate occurring in brown seaweeds inhibited release of hyaluronidase and histamine from mast cells.

Alginate was shown to be able to stimulate Toll-like receptors and activate production of cytokines (Draget and Taylor 2011).

Another possible application of alginate in pharmacology is due to its significant role as a base for the production of controlled-release drug products (Lee and Mooney 2012).

Antiallergic activities of alginic acid were shown to be a result of its suppressive effect on histidine cocarboxylase, interleukin and TNF-α production and reduce systemic anaphylaxis (Jeong et al. 2006).

The pharmacological activity of other polysaccharides from algae was also proved and described in the literature. Porphyran isolated from *Porphyratenera* was shown to be effective against different allergic responses and reduced contact hypersensitivity reaction by decreasing of IgE in Balb/c mice (Ishikara et al. 2005).

Carrageenans from *Gigartina skottsbergii* demonstrated potential activity against different strains of HSV (Smit 2004). Similar properties were observed for some agaroids from *Gracilaria corticata* (Mazumder et al. 2002).

Galactan from red seaweed *Cryptonemia crenulata* was described as a selective inhibitor of DENV-2 acting through the prevention from virus multiplication during infection (Talarico et al. 2007).

In another study, anticoagulant activity of sulfated galactans isolated from *Gellidium crinale* and *Botryocladia occidentalis* was examined. It was observed that proportion of 2,3-di-sulfated α-units in galactan chain is crucial for interaction between protease and coagulation inhibitor (Pereira et al. 2005).

Galactans from *Callophyllis variegata* were shown to possess antiviral activity against HSV and DENV (Rodriguez et al. 2005).

Matsuhiro et al. (2005) isolated galactans by aqueous extraction of *Schizymeniabinderi*. Extracted polysaccharides revealed high antiviral activity against HSV types 1 and 2. It was concluded that antiviral activity of galactans was due to the fact that polysaccharides interfere with the initial adsorption of the virus.

2.2 Pigments

Carotenoids, one of the most abundant group of molecules in algae were found to exhibit different biological activities. There is growing interest in their isolation from microalgae mainly due to their potentially beneficial characteristics for pharmacology (Crupi et al. 2013). Carotenoids isolated from algae are famous mainly for antioxidant properties. Currently, an emphasis is put on different groups of carotenoids such as fucoxanthin, astaxanthin, zeaxanthin due to the high potential for the use as pharmaceuticals. It was postulated that the application of algal carotenoids in modern pharmacology can lead to the development of cancer or cardiovascular diseases treatment (Gammone et al. 2015).

The antioxidant properties of fucoxanthin were examined in the in vitro experiments. High radical scavenging activity of isolated marine carotenoids was assumed to be a result of the presence of allenic bonds (Sachindra et al. 2007).

Fucoxanthin was presented as an efficient inhibitor of the cyclin-dependent kinase in the treatment of melanoma cells. Beneficial properties of this pigment were proved in the in vitro and in vivo experiments (Kim et al. 2013).

In another study, the same carotenoid produced by *E. bicyclis* and *Undaria pinnatifida* was shown to possess inhibitory activity against PTP1B—negative regulator of the insulin-signaling pathway (Matsuno 2001).

Among many beneficial health effects of fucoxanthin, anti-obesity properties seem to be the best examined (Apostolidis and Lee 2011). Ability of fucoxanthin to decrease glucose levels in blood led to comprehensive studies over its application in diabetes and obesity treatment (Jung et al. 2012). Fucoxanthin from *U. pinnatifida* was recognized for its inhibitory effect against pancreatic lipase (Matsumoto et al. 2010). In another study, it was demonstrated that the main health care action of fucoxanthin was based on the reduction of cardiovascular risk factors (obesity, cholesterol concentration and hypertension) (D'Orazio et al. 2012).

The antioxidative potential of astaxanthin was shown to be even higher than vitamin E and β-carotene. Pigments found mainly in microalgae such as microalgae *Haematococcus pluvialis*, *Chlorella zofingiensis*, and *Chlorococcum* sp. promotes immune response in liver and kidney diseases. Also, its extraordinary potential in the protection against cancers, diabetes and gastrointestinal diseases was proved (Yuan et al. 2011).

Another algal carotenoid—violaxanthin—was shown to be the major factor detected in dichloromethane extract from *Dunaliella tertiolecta*, which demonstrated strong antiproliferative activity against two cell lines of human mammary cancer (MCF-7 and LNCaP) (Pasquet et al. 2011). Violaxanthin was also isolated from *Chlorella ellipsoidea* and examined for its antiinflammatory activity with the use of lipopolysaccharide (LPS)—stimulated RAW 264.7 mouse macrophage cells. As the result of the test, inhibition of NO and prostaglandin E2 was revealed. It was shown that violaxanthin acts mainly on the NF-κB pathway (Soontornchaiboon et al. 2012).

In another study, hepatoprotective properties of the carotenoid-rich extract obtained from Spirulina sp. were examined on rats. Obtained results revealed that carotenoids from microalgae were characterized by the greater antihepatotoxic effect in comparison with synthetic compounds (Murthy et al. 2005).

Inhibitory effects of carotenoids on the increase of ROS levels in gastric cells induced by H_2O_2 were reported. Furthermore, the application of carotenoids led to the activation of NF-κB and interleukin 8 expressions. It was claimed that supplementation of carotenoids significantly reduced the risk of gastric cancer (Kim et al. 2011). Administration of β-carotene to OVA-immunized mice decreased serum histamine level and hence inhibited an anaphylactic response (Vo et al. 2012).

Another important pigment isolated from microalgae with well-documented pharmaceutical potential is phycoyanin. It is one of the major pigments from *Spirulina sp.* Its inhibitory activity of allergic responses, such as ear swelling, skin reactions and histamine release from mast cells was documented (Ramirez et al. 2002). Phycoyanin poses also antiinflammatory activity through enhancement of IgA antibody response and suppression of allergivIgE antibody response (Nemoto-Kawamura et al. 2004).

The possible application of phycobilin pigments, such as phycocyanin or phycoerythrin, unique for algae, in photodynamic therapy during cancer treatment was pointed out by Borowitzka (2013).

2.3 Terpenoids

Among organic compounds with high potential for the use in pharmaceuticals and isolated from algae, terpenoids should be also listed.

Methanolic extract from *Sargassum micracanthum* was shown to be rich in benzoquinone-type compounds and poses great inhibitory activity on lipid peroxidation. Strong antiviral activity against MCMV of the obtained extract was also investigated (Iwashima et al. 2005). Terpenoids extracted from *Dictyotapfaffii* were presented as potential anti-HIV-1 agents. Conducted experiments showed that *Dolabella dienetriol* can act as HIV-1 reverse transcriptase enzyme inhibitor (Crime-Santos et al. 2008).

Quinone metabolites isolated from *Sargassum sagamianum* revealed antibacterial activity against *Staphylococcus aureus* (Horie et al. 2008). Rhipocephalin, sesquiterpenoid from green algae exhibited high activity in the inhibition of phospholipase A2 from bee venom (Mayer et al. 1993).

Ji et al. (2008) investigated the possibility of the application of triterpenoids isolated from marine algae *Laurencia mariannensis* in cancer treatment. Obtained results revealed the cytotoxic effect of lauren mariannol and hydroxythyrsiferol against P-388 tumour cells. Triterpenoids extracted from *Laurencia viridis* displayed cytotoxic activity against murine leukaemia cell lines. Inhibitory activity on protein phosphatase was also determined (Souto et al. 2003).

2.4 Alkaloids

Despite the content of alkaloids in algae is relatively low in comparison with terrestrial plants, they were indicated as factors with high potential for the use in health protection. Alkaloids isolated from marine algae are known for antifungal, antioxidant and antibacterial activity. There are also some reports about the application of alkaloids as pharmaceuticals for effective neuromodulation, growth regulation and neurotransmission (Barbosa et al. 2014; De Souza et al. 2009).

The inhibition of Aβ-induced SH-SY5Y cell damage by racemosins A and B from *C. racemosa* was evaluated by Liu et al. (2013) in the in vitro experiments.

Alkaloids isolated from *Caulerpa taxifolia* (Vahl) C. Agardh. displayed inhibitory activity against PTP1B and hPTP1B (Mao et al. 2006).

2.5 Phenolic Compounds

Polyphenols, beside polysaccharides, are one of the most common secondary metabolites produced by algae. Although high content of phenolic compounds in terrestrial plants has been reported, there are fundamental differences in the chemical structure—plant molecules are derivatives of gallic and ellagic acids (Haslam and Cai 1994), whereas most frequently studied polyphenols from algae are composed of polymerized 1,3,5-trihydroxybenzene (phloroglucinol) units. Various algal polyphenols have been isolated, such as: phenolic acids, tannins, flavonoids, catechins, phlorotannins (Kadam et al. 2013); yet, scientific works concerning pharmaceuticals usually focus only on the latter group from marine brown seaweeds, particularly *Ecklonia* sp. (Singh and Bharate 2006). Beside phloroglucinol, eckol, dieckol, bieckols, phlorofucofuroeckol A and B were suggested, among other phlorotannis, for pharmacological activities (Li et al. 2009; Nagayama et al. 2008; Yoon 2008; Yoon et al. 2008).

Algal phenolic compounds are known for their strong radical scavenging effect, meaning inhibition of H_2O_2—induced DNA damage in particular (Heo and Jeon 2008). Efficacy of eckol, phlorofucofuroeckol A, dieckol, and 8,8'-bieckol—derived from *Ecklonia* species, against O_2^- and 1,1-diphenyl 1,2-picrylhydrazyl was comparable to L-ascorbic acid and α-tocopherol (Shibata et al. 2008). Moreover, *E. cava* dieckol was proved to exceptionally mitigate the results of photo-oxidative stress (Heo et al. 2009; Xie et al. 2009). Kim et al. (2006b) showed phlorotannis isolated from *E. cava* to inhibit matrix metalloproteinase enzymes responsible for chronic inflammation.

Ecklonia-derived phlorotannins were also verified for antimicrobial activity against *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Staphylococcus aureus* (Eom et al. 2008; Nagayama et al. 2002), while supplementation with phenolic compounds from *Ascophyllum nodosum* reduced the prevalence of *Escherichia coli* O157:H7 among feedlot steers (Braden et al. 2004).

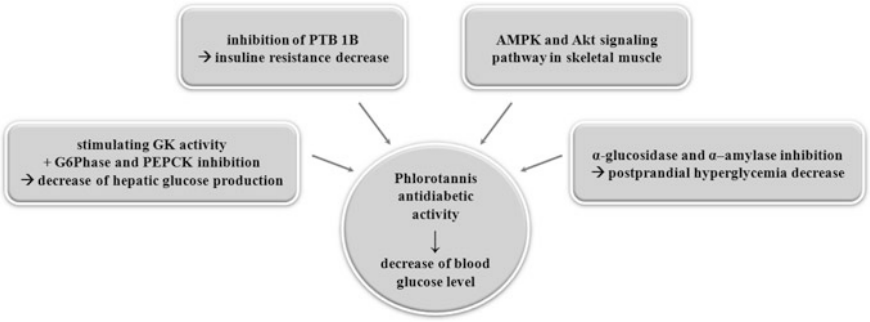

Fig. 2 Mechanisms of phlorotannis antidiabetic activity; GK—hepatic glucokinase, G6Phase—glucose-6-phosphatase, PEPCK—phosphoenolpyruvate carboxykinase, AMPK—5′ adenosine monophosphate-activated protein kinase, Akt—protein kinase B (Lee and Jeon 2013)

Antiviral effect of extracts from brown seaweeds—besides *E. cava*, such as *Dictyota pfaffi*, *Ishige okamurage*, *Peyssonelia* sp.,—were investigated against human immunodeficiency virus type-1 (HIV-1) and inhibition of HIV-1 reverse transcriptase, HIV-1 integrase and protease were observed (Lee and Jeon 2013).

Currently, a great attention is paid to examine phlorotannins for antidiabetic and anti-obesity activity. The former involves mechanisms shown in Fig. 2, while the latter is correlated with inhibition of pancreatic lipase—a key enzyme for triglyceride absorption in the small intestine (Lowe 1994, 2002).

The most recent trend to phlorotannis from *Ecklonia maxima* and *Ishigeo kamurae* pharmacological application is to inhibit acetylcholinesterase to protect the neurons from neurodegeneration—especially Alzheimer's disease—by the enhancement of cholinergic neurotransmission in the brain and, simultaneously, ß-amyloid formationdecrease (Hodges 2006; Kannan et al. 2013; Yoon et al. 2009). Yoon et al. (2008) showed *E. maxima* extract to affect both acetyl and butylcholinesterase, while the activity of phenolic compounds from *Sargassum* species were proved against cholinesterase (Choi et al. 2007; Kusumi et al. 1979a, b; Ryu et al. 2003).

3 Peptides and Proteins

Due to intensive studies on the correlation between exposure to oxidative stress and chronic diseases, algal peptides as stress-response compounds have been investigated (Bondu et al. 2015). In vitro examination showed components derived from both micro—*Chlorella vulgaris*, *C. ellipsoidea* and *Navivulla incerta* (Kang et al. 2011, 2012; Ko et al. 2012; Sheih et al. 2009) and macroalgae—*Solieria chordalis*, *Palmaria palmata*, *Ulva lactuca* and *Saccharina longicruris*, to have activity against radicals, particularly hydroxyl radicals (Fan et al. 2014).

Most peptides showed activation when released from the parent protein molecule by hydrolysis (Fan et al. 2014). At the same time, the use of peptides is limited by their susceptible to degradation in the gastrointestinal tract and chemical modification or encapsulation of biomolecules is required (Shen et al. 2010; Walsh et al. 2004; Wang and Zhang 2013). Regardless of the application issues, algal peptides are also tested for blood pressure control, antiatherosclerotic and anticancer activity.

Cha et al. (2006) verified peptides isolated from *Ecklonia cava* for affecting hypertensive rats by ACE inhibition. The same mechanism of decreasing blood pressure was observed for microalgal peptides (Ko et al. 2012; Samarakoon et al. 2013). *Chlorella* and *Spirulina*-derived peptides were also reported to inhibit the production of adhesion molecules, and thus prevent atherogenesis (Shih et al. 2013; Vo and Kim 2013).

In the work of Zhang and Zhang (2013), anti-tumour peptide from *Spirulina platensis* with activity against human liver cancer MCF-7 and HepG2 cells is reported. On the other hand, *Chlorella* sp. components showed cytotoxicity towards both HepG2 cells and gastric cancer (AGS cells), their effectiveness was, however, lower by 80% (Sheih et al. 2010; Wang and Zhang 2013).

4 Fatty Acids

Algae are proved to surpass terrestrial plants in content of essential fatty acids, which, besides being well known dietary supplements of mainly antiatherosclerotic and immune-enhancing effect (Kay 1991; Khotimchenko 1993; Sanchez-Machado et al. 2002), show antiallergic activity by limiting the production and/or the release of allergic response mediator. Polyunsaturated fatty acids—stearidonic acid (C18:4, n-3) and hexadecatetraenoic acid (C16:4, n-3)—from *Undaria pinnatifida* and *Ulva pertusa* effectively decreased content of leukotriene B4, leukotriene C4 and 5-hydroxyeicosatetraenoic acid in MC/9 mouse mast cells (Ishihara et al. 1998). Histamine production was efficiently inhibited by treatment of RBL-2H3 cells with both α- and γ-linolenic acid (C18:3, n-3 and n-6, respectively) (Gueck et al. 2003; Kawasaki et al. 1994). Gueck et al. (2003, 2004) verified α- and γ-linolenic acid and docosahexaenoic acid (C22:6, n-3) to affect PGE2 production and histamine release in the canine mastocytoma cell line C2.

5 Conclusions

Algae constitute an abundant source of bioactive compounds, which have a great potential to be used as pharmaceuticals. Currently, the growing interest is put on the application of different algal compounds in the civilization diseases treatment. The market for pharmaceuticals based on compounds of natural origin is growing

worldwide. Still untapped reservoir of chemically active compounds and potential in the field of pharmaceuticals imply requirement of increased screening algae for healthcare chemicals. Equally important is development of isolation methods.

Acknowledgments This project is financed in the framework of a grant entitled *Innovative technology of seaweed extracts—components of fertilizers, feed and cosmetics* (PBS/1/A1/2/2012) attributed by The National Centre for Research and Development in Poland.

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Production of Primary and Secondary Metabolites Using Algae

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Abstract Over the past few decades, there has been an increase in the number of research studies focused on all aspects of the ecology, physiology, biochemistry, cell biology, molecular biology, systematics and uses of algae. This chapter will provide an overview of the potential human health advantages associated with the use of algae as a source of high-value products, especially focused on those metabolites with biological activities and potential therapeutic applications in the pharmaceutical and food industries. Moreover, the production of polyphenols by marine microalgae will be also considered, as well their impact on the biogeochemical cycles of trace metals and the phytoplankton implications. These data will support as a baseline for future research in wastewater and marine environments.

1 Introduction

The interest of algae, cyanobacteria, and microalgae increased in the last decades, achieving a total number of publications of 3,586 (the 1960s) and 56,313 in the 2000s (Fig. 1), according to Scopus database. This increase is due to several factors: improvement of technology, the increased demand for healthy and functional foods, pharmacological properties of algal metabolites for disease prevention and treatment and, algae biofuels research in order to provide a viable alternative to fossil fuels, among many others.

The main subject areas in which these documents are issued within the last three decades are Agricultural and Biological Sciences, Biochemistry, Genetics and Molecular Biology, Environmental and Planetary Sciences and Earth and Planetary Sciences (Fig. 2). The percentage of scientific publications reaches values as high as 36.2% (2001–2010), 42.0% (1991–2000), and 46.4% (1981–1990) when subject

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B.N. Tripathi and D. Kumar (eds.), *Prospects and Challenges in Algal Biotechnology*, DOI 10.1007/978-981-10-1950-0_12

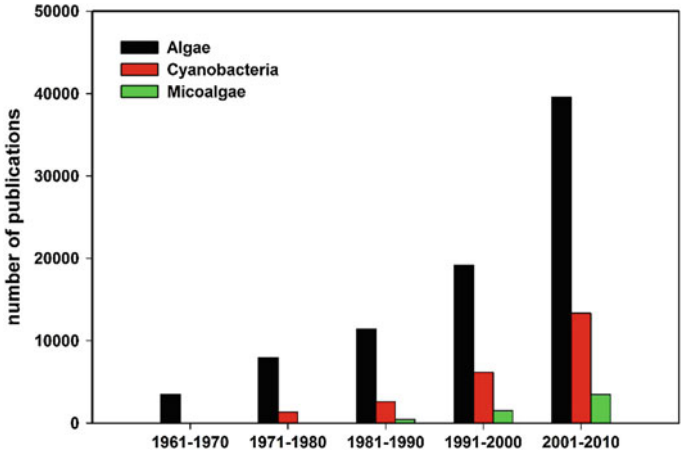

Fig. 1 Evolution of the number of scientific documents reported per decade

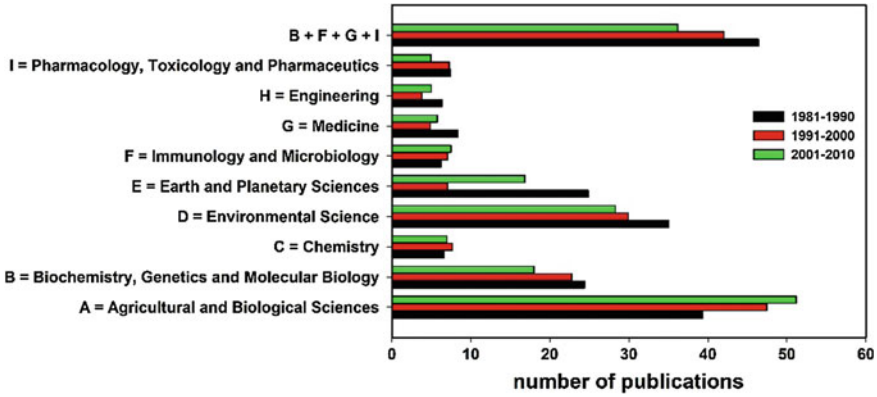

Fig. 2 Percentages of documents per subject area according to Scopus database

areas related to human and animal health sciences are considered. Within these areas, we can find toxicology, cell and molecular biology, biomedical research, basic clinical and pharmaceutical microbiology, pharmaceutical biotechnology, medicinal chemistry, phytochemistry, and nutraceuticals.

The extensive research on extracting drugs from the sea organisms began in the mid-1970s. Since 1975, the reported applications of these drugs isolated from algae are numerous (Priyadarshani and Rath 2012). These studies have clearly demonstrated that algae represent valuable sources of a wide variety of compounds with different potential applications in food, cosmetic and pharmaceutical industries (Li et al. 2014; Machu et al. 2015). Developing new technologies after the 1980s has led to extract new metabolites from algae cultures and to determine the chemical structure of 15,000 novel compounds, decreasing the biomass needed from

kilograms to grams (Borowitzka 1995). This also makes possible to increase the number of newly studied species, especially for microalgae.

Algae range from unicellular organisms such as diatoms to seaweeds extending over 30 m long. Diatoms have been widely investigated because they are a diverse group of microalgae (250 orders and more than 10^5 species) and the sole ones with a siliceous cell wall (Norton et al. 1996). Diatoms contribute as much as 25% of the global primary productivity (Scala and Bowler 2001). A high variety of phytoplankton species has been described (40,000 species), being 680 species of marine algae divided in Rhodophyta, Phaeophyta, Chlorophyta (Boopathy and Kathiresan 2010).

Marine algae can be considered an arsenal of metabolites with pharmaceutical potential, including anticancer, antitumor, antioxidant, antiobesity, neuroprotective, antimicrobial, antinociceptive, anti-inflammatory, and antiangiogenic activities (Cornish and Garbary 2010; Gupta and Abu-Ghannam 2011; Pangestuti and Kim 2011). Microalgae are able to produce highly bioactive compounds extracted from the marine environments (Shimizu 1996). The antibacterial activity of substances excreted by an aquatic microalga was first reported for *Chlorella vulgaris* (Pratt and Fong 1940). Currently, algae have attracted attention because they are biological systems capable of using solar energy to produce a large number of active metabolites via the photosynthetic process with the highest efficiency (Shalaby 2011). Blue-green algae produce a number of highly active antitumor compounds (Shimizu 2000).

The focus in this chapter is placed on the main classes of algal metabolites that could be of medicinal, nutritional and pharmaceutical value. Seaweeds may become a suitable natural source of bioactive compounds reported to possess strong antiviral, antitumor and anticancer properties, among others. Here, we discuss the pharmaceutical, health and research potential of different primary and secondary metabolites present in algae, with a focus on those from microalgae and paying special attention to the polyphenolic compounds and the involvement of these compounds in protection cell mechanisms in conditions of mental stress.

2 Primary Metabolites

Primary metabolism represents an essential biogeochemical pathway directly involved in cell growth and reproduction. The most important primary metabolites are carbohydrates, lipids, and proteins (Wen et al. 2015).

Lipids

Lipids content of algal cells can achieve 90% of dry weight (Metting et al. 1996). These lipids consist in phospholipids, glycolipids, non-polar glycerolipids, saturated and unsaturated fatty acids (PUFA) (Kumari et al. 2013). Within the group of PUFA, linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, and

docosahexaenoic acid have great interest for many diseases as cancer, cardiovascular, diabetes, inflammatory, asthma, etc. (Andersen et al. 2008; Priyadarshani and Rath 2012).

Crude lipids have been extracted from a wide group of algal species (*Laurencia popillose, Galaxoura cylindriea, Ulva fasciata, Taonia atomaria,* and *Dilophys fasciola*), but the group of *Dunaliella, Chlorella,* and *Spirulina* species are the most interesting in terms of high concentration of lipids (El Baz et al. 2002; Abd El Baky et al. 2014a, b). On the other hand, *Phaeodactylum tricornutum* (marine diatom) is also considered the main producer of eicosapentaenoic acid (EPA) (Grima et al. 2003) and *Nitzschia inconspicua* (marine diatom) produces high levels of arachidonic acid, an essential polyunsaturated fatty acid (Chu et al. 1994) under deficiency in linoleic acid.

In general, crude lipids of several algal species such as *Laurencia popillose, Galaxoura cylindriea, Ulva fasciata, Taonia atomaria,* and *Dilophys fasciola*) showed different biological properties as antitumor, antioxidant, antimicrobial and antivirus with various degrees (Abd El Baky et. 2014a, b; Ghazala et al. 2010). PUFA microalgae-derived such as γ-linolenic acid, arachidonic acid, EPA and docosahexaenoic acid (DHA) are widely used as nutritional supplements, pharmaceutical, and functional food and their roles in promoting human health have been extensively studied (Borowitzka 2013; Michalak and Chojnacka 2015; Priyadarshani and Rath 2012).

Polyunsaturated fatty acids, phenolic and flavonoids from *T. atomaria* and *L. papillose* have shown an inhibitory effect against human hepatocarcinoma and human breast carcinoma. These complex lipids are usually rare in terrestrial plants but are present in relatively large amounts in some species of marine algae (Abd El Baky et al. 2014a, b; Castro et al. 2016; Harwood and Guschina 2009).

Proteins

Marine algae have high protein content, up to 70% of the dry weight, depending on the season and the species (Abreu et al. 2014; Fan et al. 2014). According to Becker (2007), *Arthrospira maxima* (cyanobacteria with 60–71% proteins) is one of the most important microalgae in this sense. Currently, marine algae proteins have attracted great interest as a source of bioactive peptides for therapeutic uses (Kim and Kang 2011). Several studies have isolated various algal peptides possessing anti-infective activities (Kang et al. 2015a, b). Depending on the amino acid sequence, biopeptides isolated from marine algae may be associated with different biological functions, including antioxidant, anticancer, antihypertensive, anti-atherosclerotic, and immunomodulatory effects (Fan et al. 2014).

Carbohydrates

Carbohydrates can be found as glucose, sugars and other polysaccharides in microalgae, making relevant the production of carbohydrates because of their high overall digestibility (Becker 2004). In this sense, carbohydrates compounds may

achieve from 10% (*Scenedesmus obliquus*) to 57% (*Porphyridium cruentum*) of dry biomass (Becker 2004).

Among marine biologically active metabolites, polyphenols and polysaccharides have shown more effective antioxidant and anticancer activities (Farvin and Jacobsen 2013; Li et al. 2014). In the last decades, the study of polysaccharides from marine algae has gained renewed interest for their many valuable biological properties and therapeutic applications (Barahona et al. 2014; Kang et al. 2015a, b). Hossain et al. (2005) suggested that algal glycolipids such as digalactosyl diacylglycerol and sulfoquinovosyl diacylglycerol combined with sodium butyrate might be used as potential colon cancer chemotherapy agents. *Spirulina platensis* accumulate a high amount of sulfated polysaccharides with biological activities as an anticoagulant, anticancer, antiviral, antimicrobial, and antioxidant (Abd El Baky et al. 2013). In addition, red algae are important sources of sulfated polysaccharides which show antiviral activities in human infectious diseases by viruses (Damonte et al. 1994; Witvrouw et al. 1994). The inhibitory effect is mostly due to the interaction with the positive charges on the viruses cell surfaces, preventing the penetration into the host cells (Ehresmann et al. 1979) *Aghardhiella tenera* and *Nothogenia fastigiata* are characteristics because of their capacity to produce polysaccharides with low cytoxoxic activities (de Clercq 1996). Another important use of polysaccharides is the antiviral action against two enveloped rhabdovirusees with high economic impacts, such as hemorrhagic septicemia virus and the African swine fever virus. Some algal polysaccharides and fibers are also able to reduced cholesterol absorption in the gut, such as alginate, carrageenan, funoran, fucoidan, laminaran, porphyrin and ulvan (Kiriyama et al. 1969; Lamela et al. 1989; Panlasigui et al. 2003). Accordingly, the production of polysaccharides seems to be a potential industrial option against viral therapy.

Several reviews focused on algae-derived polysaccharides (alginate, galactan, laminaran, and naviculan) with different antiviral activities and mechanisms of action have been recently reported (Ahmadi et al. 2015; Zubia et al. 2008).

3 Induction of Lipids, Peptides, and Carbohydrates of Commercial Value

Algae differ in their tolerance and adaptability to the environmental conditions. Under stress conditions, algae accumulate elevated amounts of some metabolites for protection and recovery from injury. The growth of *Botryococcus braunii* (race 'A') and production of its constituents, hydrocarbon, carbohydrate, fatty acid, and carotenoids were influenced by different levels of salinity (Ranga Rao et al. 2007a, b). Different microalgae species react to different stresses by producing different fatty acids or by altering their composition of fatty acids. The exact combination of induction stresses that provides optimum lipid productivity in a large-scale commercial cultivation system for biodiesel production will differ for every microalgae

strain and depends on nutrient supply, environmental and climatic conditions (Mata et al. 2010; Sharma et al. 2012). Several studies have focused on the increase of lipid productivity by the cultivation of microalgae with different levels of CO_2, (Yoo et al. 2010; Ranga Rao et al. 2007a, b), nitrogen concentration and temperature, to mitigate CO_2 due to its C-fixation ability and for production of biodiesel. In algae and cyanobacteria, several protection mechanisms against ultraviolet stress have been reported (Xue 2005; Lee et al. 2014; Hartmann et al. 2015). *Spirulina platensis* produced relevant amounts of sulfated polysaccharides as adaptation mechanisms to nitrogen concentration (Abd El Baky et al. 2013).

4 Several Secondary Metabolites

Secondary metabolites have gained great attention over the last 50 years because they showed a wide variety of pharmacological uses. These molecules are mainly involved in the adaptation of organisms to their environment (Bourgaud et al. 2001).

The metabolism is the sum of all the biochemical processes that take place in an organism. Secondary metabolic pathways produce a huge number of compounds: alkaloids, carotenoids, phenolic compounds, lignin, phytosterols, and others (Hussain et al. 2012). The major metabolic reactions that occur in algae are unique and produce unique secondary metabolites: mechanisms of light harvesting, carbon acquisition and aspects of nitrogen (N) and sulfur (S) assimilation, growth in extreme environments, such as nutrient limitation and exposure to extremes of visible and UV light (Beardal and Ravenm 2012). It has been reported that algal species cultivated under stress conditions produce relevant amounts of metabolites for pharmaceutical uses (Leu and Boussiba 2014; Yu et al. 2015).

Carotenoids

Microalgae are rich in carotenoids, being especially high in *Dunaliella* species where it is up to 14% of dry weight (Spolaore et al. 2006). They are over 400 known carotenoids but only one small fraction is commercially produced, highlighting β-carotene, astaxanthin, lutein, zeaxanthin, lycopene, and bixin (Radmer 1996; del Campo et al. 2000). The green flagellate microalgae *Dunaliella salina* is the most important species in the production of β-carotene (Metting 1996). In general, these carotenoids are a strong antioxidant, making them highly relevant in terms of industrial production.

Algal carotenoids are involved in different functions: in photosynthesis, as intermediates of carotenogenesis or as accumulated carotenoids (Takaichi 2011). They have been found to protect against several malfunctions and diseases triggered by oxidative stress (Abd El Baky 2003; Fassett and Coombes 2011).

Fucoxanthin is a marine carotenoid widely distributed in microalgae and brown macroalgae, involved in a multitude of molecular and cellular processes, showing a

protective role and anti-proliferative behavior in several types of cancer (Kotake-Nara 2005; Mikami and Hosokawa 2013; Satomi 2012). In fact, the regular consumption of seaweeds containing high quantities of fucoxanthin is believed to be the reason for the longevity of certain populations (Kumar et al. 2013).

Astaxanthin, a rare ketocarotenoid synthesized only by limited numbers of organisms, has therapeutic applications, for example, against free radical-associated diseases like oral, colon, and liver cancers, cardiovascular diseases, and degenerative eye diseases (Han et al. 2013). Several reviews focused on carotenoids from algae have recently been published (Jin and Melis 2003; Guedes et al. 2011; Mezzomo and Ferreira 2016; Takaichi 2011). Moreover, astaxanthin is a main compound in the salmon feed industry with more than US$200 million (Hejazi and Wijffels 2004).

Phycobiliproteins

The most common phycobiliproteins in the industry are phycoerythrin and phycocyanin that can be produced by *Arthrospira* species and *Porphyridium* species (Viskari and Colyer 2003; Roman et al. 2002). The application of phycobiliproteins is focused in health-promoting properties, such as food pigment. On the other hand, phycobiliproteins are interesting in clinical applications due to their high molar absorbance coefficients, high fluorescence quantum yield, and high photostability. In this sense, phycobiliproteins can be used as labels in fluorescence medical diagnostics (Roman et al. 2002).

Phytosterols

Recently has been published a review focused on microalgae-derived phytosterols applications in functional food and pharmaceutical industries (Luo et al. 2015). Phytosterols are important structural components of the cellular membrane and have important functions in regulating membrane fluidity and permeability. They also exist as hormones or hormonal precursors and are involved in signal transduction in the organisms. In addition, phytosterols reduce the level of cholesterol in blood helping to prevent cardiovascular disorders (Ras et al. 2013; Gylling et al. 2014).

Phenolic Compounds

Waterman and Mole (1994) defined polyphenolic secondary metabolites as a large and diverse group of chemical compounds, which are common both in terrestrial plants and in aquatic macrophytes (without citing microalgae). The term phenolic includes more than 8000 naturally compounds with one common structural feature, a phenol. The phenolic compounds are divided into two groups according to the number of phenol subunits: simple phenols and polyphenols (with at least two phenol subunits) (Leopoldini et al. 2011), and tannins were described as compounds possessing three or more phenol subunits (Clifford 1999). Polyphenols are

primarily recognized for their antioxidant capability toward free radicals normally produced by cells metabolism or in response to external factors. In living systems under stress, the excessive generation of hydroxyl radical ($OH^{\bullet}$) and other highly reactive oxygen species (ROS) produce oxidative damage through the reaction of these species with almost every cellular biomolecules including DNA. Many studies on pharmacological research have evidenced that oxidative stress and increased amounts of free radicals are features of chronic diseases including cancer (Klaunig and Kamendulis 2004), aging and neurodegenerative diseases such as Alzheimer's and Parkinson's (Nunomura et al. 2006; Wood-Kaczmar et al. 2006) and cardiovascular diseases such as atherosclerosis (Siti et al. 2015). They are the primary causes of cell death and tissue damage resulting from a heart attack and stroke (Perron and Brumaghim 2009). Phenolic compounds are radical scavengers and inhibit metal-mediated oxyradical formation preventing various processes of oxidative stress considered the origin of the above-cited diseases (Jomova and Valkoa 2011; Zhaoa et al. 2005; Aprioku 2013). Therefore, polyphenols display a number of pharmacological, medicinal and biochemical properties extensively reviewed: they present antiviral, anti-inflammatory, antibacterial, and antihistamine activities, cardiovascular effects and are implicated in the prevention of neurodegenerative diseases (Brit et al. 2001; Dai and Mumper 2010; Graf et al. 2005; Hertog et al. 1993; Mandel et al. 2006; Lambert and Yang 2003; Pandey and Rizvi 2009; Quideau et al. 2011).

Several mechanisms have been proposed for polyphenol prevention of oxidative stress: they are able to inhibit free radicals according to the hydrogen atom transfer and to the single electron transfer mechanisms. In the first one, the bond dissociation enthalpy of the phenolic O–H bond is an important parameter in evaluating the antioxidant action, while in the second the ionization potential is the most significant parameter for the scavenging activity evaluation. These mechanisms often take place simultaneously and depend on the pH (Leopoldini 2011; Perron and Brumaghim 2009). Polyphenols are also capable of chelating metal ions leading to stable complex compounds and avoiding them to take part in the reactions generating free radicals (Flora 2009).

Among phenolic compounds, phlorotannins (polymers of phloroglucinol units linked to each other in various ways) have only been detected in brown algae (Koivikko et al. 2007; Li et al. 2011; Ragan and Glombitza 1986) such as seaweeds *Ecklonia* sp., which have provided various phlorotannins with diverse biological activities (Kang et al. 2004; Kim et al. 2006: Shin et al. 2014; Wijesinghe et al. 2011). Consumption of phlorotannin-rich algae *Fucus distichus* and *Ecklonia cava* may be useful for the treatment of diabetes (Kellogg et al. 2014; Lee and Jeon 2015). Phlorotannin dieckol, isolated from the edible brown algae *Ecklonia cava*, suppresses ovarian cancer cell growth (Ahn et al. 2015) and shows antithrombotic activity among others, useful in the development of agents for the anticoagulation (Kim et al. 2012).

On the other hand, only a few references have dealt with the beneficial effects of phenolic compounds from microalgae in human health. Despite marine microalgae are known to produce numerous useful products, have attracted little attention in the search of polyphenolic compounds. Recently, several methodologies for identification and quantification of polyphenolic compounds from microalgae extracts have been reported (Rico et al. 2013; López et al. 2015). These publications are focused on the implications of polyphenols in microalgae growing under metal stress paying special attention to the influence of Cu(II) and Fe(III) metals on the cells and exudated phenolic profiles of the marine green microalgae *Dunaliella tertiolecta* and the marine diatoms *Phaeodactylum tricornutum*. In these studies, the authors measured the concentration of gallic acid, protocatechuic acid, (+) catechin, vanilic acid, (−) epicatechin, syringic acid, gentisic acid, caffeic acid, coumaric acid, ferulic acid, rutin, myricetin and quercetin.

Significant differences in the phenolic profiles were found depending on the metal added to the seawater indicating the involvement of polyphenols in the microalgae cellular response under a high level of Cu(II) and Fe(III) in natural seawater. The presence of these polyphenols was also measured in the control experiments (without stress conditions) and their concentrations, both in solution and in cells, were affected by the stress caused by the addition of metals. The great increase in phenolic compounds exuded by the cells at the highest copper concentration may reflect the involvement of these compounds in protection in conditions of copper toxicity (Fig. 3).

Cells exposed to copper excreted a larger amount of polyphenols as a protective mechanism to alleviate the toxicity of the copper in the solution: these phenolic compounds are implicated countering metal toxicity at the membrane surface and slowing down the toxicity of metals in the extracellular media.

Iron is an essential mineral for all forms of life. Excess of iron results in cellular damage due to the proclivity of this transition metal to generate reactive oxygen species through Fenton chemistry, being necessary a stringent control of iron atoms once they are inside the cell (Foley and Simeonov 2012). In fact, diatom *Phaeodactylum tricornutum* exposed to high levels of iron produced relevant amounts of phenolic compounds in the cells (Rico et al. 2013). By other hand, microorganisms have developed pathways to synthesize, secrete, and retrieve small molecule chelators that display an unprecedented affinity for ferric and ferrous ions (Foley and Simeonov 2012). The concentration of phenolic compounds exuded by diatom *Phaeodactylum tricornutum* cultivated in seawater enriched with iron increased from 24 nmol L^{-1} (control) to 28 nmol L^{-1} because polyphenols are potent metal ion chelators (Wang et al. 2009). In addition, phenolic compounds exuded from microalgae, such as sinapic acid and (+) catechin, have shown also an influence in iron redox chemistry by favoring the persistence of Fe(II) for the requirements of the cells (Santana-Casiano et al. 2014). These compounds favored reduction of Fe (III) to Fe (II), which is a pH-dependent process, being the percentage of Fe (II) regenerated always higher in the presence of (−) catechin than in the presence of sinapic acid (Fig. 4).

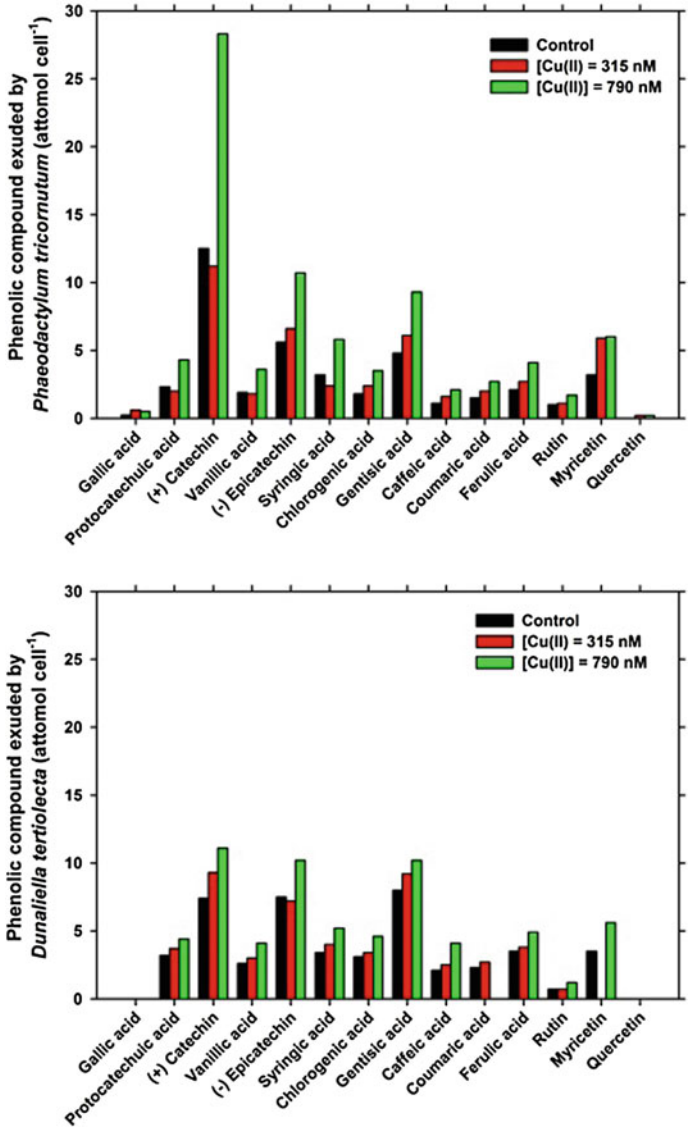

Fig. 3 Phenolic compounds exuded by *Phaeodactylum tricornutum* and *Dunaliella tertiolecta* cultured in seawater, during 8 days without metals (control) and after Cu(II) additions. Data collected from Rico et al. (2013) and Lopez et al. (2015)

The extracts of *Dunaliella tertiolecta* and *Phaeodactylum tricornutum* showed antioxidant activity and high amounts of phenolics confirming their pharmaceutical and medicinal value.

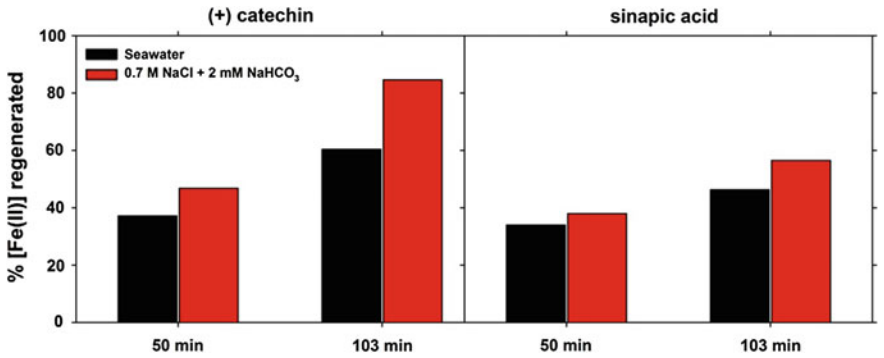

Fig. 4 Percentage of regenerated Fe (II) from initial concentration of 200 nM Fe(III) at pH = 6.00 in seawater and in 0.7 M NaCl (+2 mM $NaHCO_3$). Data from Santana-Casiano et al. (2014)

Acknowledgements Authors thank the Project CTM2014-52342-P given by the Ministerio de Economia y Competitividad from Spain. Aridane G. Gonzalez thanks the French "Agence Nationale de la Recherche" through the "Laboratoire d'Excellence" LabexMER (ANR-10-LABX-19-01) program, and co-funded by a grant from the French government through the "Investissements d'Avenir" and the Brittany Region.

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