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Microalgae-based Systems for Carbon Dioxide Sequestration and Industrial Biorefineries

Eduardo Jacob-Lopes[1] and Telma Teixeira Franco[2]
[1]*School of Agricultural Engineering, Federal University of Pelotas, UFPel, 96010-900, Pelotas-RS,*
[2]*School of Chemical Engineering, State University of Campinas, UNICAMP, P.O. Box 6066, 13083-970, Campinas-SP, Brazil*

1. Introduction

The bulk of the evidence indicating that global climatic alterations occur as a result of increasing concentrations of greenhouse gases in the atmosphere has created pressure to develop strategies to reduce these changes (IPCC, 2001). Carbon dioxide is considered to be the main gas of the greenhouse effect, both in terms of emission and its climate-altering potential.

In 1997, the signatory countries of the Kyoto Protocol agreed to reduce CO_2 emissions in an agreement that established the need to develop carbon dioxide sequestering processes. Thus the various technologies available for carbon capture and storage need to be evaluated from the point of view of obtaining carbon credits, aiming to stabilize emissions of this pollutant (UNFCCC, 1997). In addition to technologies available for immediate use, other CO_2 capture methods are being developed for application in the near future. The choice of these methodologies will depend on factors such as cost, capture capacity, environmental impact and the speed with which the technology can be introduced in addition to social factors such as public acceptance (IPCC, 2007a).

In this context, the use of biotechnological processes for carbon dioxide biofixation is considered viable for reducing emissions of this pollutant. These processes are based on the use of reactors used to develop photosynthetic reactions in which microalgae are used as biocatalysts in a series of biochemical reactions responsible for the conversion of CO_2 into photosynthetic metabolic products (Jacob-Lopes et al., 2010). With this in mind, the objectives of this present chapter are to present an overview of a potential technology for carbon dioxide transformation into biomolecules and to describe the current state of the art in the biological conversion of CO_2 in photobioreactors thereby facilitating worldwide advances in this research area.

2. Carbon dioxide emissions

Global monitoring of atmospheric CO_2 concentration during the last century indicated an increase in carbon dioxide concentration from 295ppm in 1900 to 377ppm in 2004,

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representing an increase of 27.8% (Thitakamol et al., 2007). On a global basis, it is estimated that more than 25 $GtCO_2$ are emitted annually as a result of burning fossil fuels. The magnitude of the influence of human activities on the biological carbon cycles suggests the need for high managerial levels and the mitigation of emissions of this compound into the atmosphere (IPCC, 2007b).

Sources of carbon dioxide emission can be classified as stationary, mobile or natural. The industrial processes most contributing to increasing atmospheric CO_2 concentrations consist of electrical energy generating plants, hydrogen and ammonia production plants, cement factories, and fermentative and chemical oxidation processes. In addition to the carbon dioxide emitted industrially, the CO_2 generated in residences, buildings and commercial complexes also contributes to the stationary emissions, as do forest and agricultural fires. The mobile emission sources mainly consist of the carbon dioxide generated by passenger and cargo transport including cars, trucks, buses, planes, trains and ships. Human and animal metabolism, plant and animal degradation and volcanic and oceanic activities are the main natural carbon dioxide sources. Sources of anthropogenic emissions include stationary and mobile sources but exclude the natural sources (Song, 2006).

Microalgae-based systems are restricted to the use of stationary industrial emissions. Sources of high purity CO_2 emission at reduced temperatures should be identified and the photobioreactors adapted to these conditions (Francisco et al., 2010).

3. Microalgae

Current taxonomic concepts and standards classify microalgae into groups as diatoms, chlorophyceae and cyanobacteria (Anand, 1998).

Photosynthesis is the main metabolic model of the microalgae, a process that had a central role in the rise in the oxygen level of the terrestrial atmosphere during the evolution of the current biosphere (Schmetterer, 1994). Nevertheless these microorganisms have great versatility in the maintenance of their structures, using different energy metabolisms such as respiration and nitrogen fixation (Demeyer et al., 1982; Grossman et al., 1994).

Some genera of microalgae have high concentrations of pigments, including chlorophyll *a*, considered essential for photosynthesis. Another two pigment classes involved in light energy capture are the carotenoids and phycobilins. The carotenoids are red, orange or yellow lipid-soluble pigments, found in association with chlorophyll *a*. The third class of accessory pigments is the phycobilins: phycocyanin, a blue pigment present in microalgae, and phycoerythrin, a red pigment sometimes absent (Fay, 1983). In addition to these pigments, these microorganisms have a highly developed intracytoplasmatic system, indicating photosynthesis as the preferred metabolic pathway.

The microalgae are capable of using free CO_2 and bicarbonate ions as a source of inorganic carbon during photosynthesis, transporting them across the fine plasmatic membrane where they accumulate in the cell as an inorganic carbon reservoir for photosynthesis. The bicarbonate is converted into CO_2 by the enzyme carbonic anhydrase (Zak et al., 2001; Badger & Price, 2003).

The main characteristic of photosynthesis, first elucidated in algae and higher plants, can also be applied to the microalgae, although there are some aspects specific to some microalgae. The spectral light absorption characteristic of these strains is different from that of the other photosynthetic organisms, since high photosynthetic activity rates are measured not only in the spectral region from 665 to 680nm, where the light is better absorbed by

chlorophyll *a*, but also from about 620nm to 560nm, where phycocyanin and phycoerythrin respectively absorb light effectively. This shows that the light absorbed by the phycobiliproteins is used by these microalgae as efficiently as light absorbed by chlorophyll, suggesting a very high photosynthetic activity by these microorganisms (Campbell et al., 1998).

3.1 Photosynthetic metabolism

Photosynthesis is characterized by a two-stage mechanism: a photochemical reaction and a carbon fixation reaction. In this way, carbon dioxide is incorporated into ribulose 1,5 diphosphate (rubisco) energy being required during the catalytic reaction of the primary enzyme rubisco carboxylase. The reaction product is broken into three carbon molecules, phosphoglyceric acid (PGA) and the reduction of the PGA caused by the electron transporter NADPH (nicotinamide adenine dinucleotide phosphate) leads to the production of a series of intermediary phosphorylated sugars and finally to glucose. This sequence of metabolic transformations is known as the Calvin-Benson cycle (Calvin and Benson, 1948).

Carbon dioxide fixation is not directly light dependent and thus the process is called the photosynthetic dark reaction. The demands for energy in the form of ATP and NADPH translate the transformations of the Calvin-Benson cycle, entirely dependent on the photochemical reaction, which occurs in the tilacoid or intracytoplasmatic membrane (Campbell et al., 1998). In this stage the light energy is absorbed by the highly organized structures of the photosynthetic pigments and electron transporters, known as photosystems I and II, thus exciting the chlorophyll *a* molecule. This leads to an explosion of excited electrons and their flow determines the redox potential gradient, which results in the formation of strongly electronegative electron transporters such as ferridoxin and NADPH. Part of the energy liberated is incorporated into ATP in the phosphorylation process during electron transport. The last electron source for photosynthesis is H_2O, which gives up hydrogen atoms and electrons during the photolysis process, or Hill's reaction, and releases O_2, the product of photosynthesis by microalgae and green plants (Fromme et al., 2006).

Although carboxylation by rubisco is the main CO_2 incorporation pathway in microalgae under optimum photosynthesizing conditions, this is not the only carbon dioxide fixation pathway. The carboxylation of phosphenol pyruvate, catalyzed by the enzyme phosphenol pyruvate carboxylase, is another CO_2 fixation pathway. Oxaloacetate is easily converted into C_4 dicarboxylic acids, for example into malate or citrate, and subsequently into amino acids such as aspartate or glutamate. This pathway, left over from the C_4 dicarboxylic acid pathway in higher plants, complements the pentose phosphate-reducing pathway in microalgae. The presence of two carboxylation systems, operating in parallel, could represent an important adaptation of the microalgae to sharp environmental changes. Under limited light conditions, carbon assimilation is preferentially channeled in the direction of the synthesis of amino acids and other essential cell constituents, but under saturated light conditions, sugars and starch are formed via the pentose phosphate-reducing pathway. This indicates that with intense illumination, the CO_2 fixation rate can exceed the rate of nitrogen assimilation and, thus, the excess carbon and energy derived from photosynthesis are stored in the form of glycogen (Fay, 1983; Campbell et al., 1998; Zak et al., 2001).

The dark endogenous metabolism serves mainly as an agent for the photosynthetic and biosynthetic mechanisms for the subsequent active light period. Glycogen is the main reserve product, which can support limited dark metabolism and provide the energy

maintenance required for essential cell processes in the dark. It is first converted into glucose-6-phosphate, which is then metabolized via the respiratory pathways (Fay, 1983). Although enzymes from the glycolytic pathway have been identified in microalgae, they show extremely low activity. The energy metabolism of the dark metabolism of the microalgae is distinctly dependent on O_2 and its main pathway is the pentose-6-phosphate oxidative cycle (Schmetterer, 1994).

3.2 Carbon concentration mechanisms in microalgae

The way in which the different species of microalgae adapt to a wide range of carbon dioxide concentrations is related to an essential biophysical mechanism denominated the carbon concentration mechanism (CCM), which concentrates the carbon dioxide at the photosynthetic carboxylation sites. This mechanism corresponds to complex metabolic pathways, since different forms of inorganic carbon are involved in these biological processes (Jaiswal & Kashyap, 2002).

The function of the carbon concentration mechanism is to raise the intracellular inorganic carbon levels, compensating for limitations in the carbon dioxide supply that could reduce the photosynthetic rates. This mechanism is responsible for pumping CO_2 to the carboxylation sites (Falkowski, 1997).

Microalgae are capable of using three different inorganic carbon assimilation pathways: (i) direct carbon dioxide assimilation via the plasmatic membrane; (ii) the use of bicarbonate by inducing the enzyme carbonic anhydrase, which converts the HCO_3^- into CO_2; and (iii) direct transport of bicarbonate via the plasmatic membrane. The enzymes carbonic anhydrase and ribulose 1,5 bisphosphate carboxylase/dehydrogenase (rubisco) are responsible for the biocatalysis of these reactions, in which the enzyme carbonic anhydrase converts bicarbonate into carbon dioxide, and rubisco uses this compound as a substrate to produce phosphoglycerate. The rate of this reaction may be slow due to limited carbon dioxide production. Thus the elevated efficiency of the enzyme carbonic anhydrase, capable of increasing the intracellular carbon dioxide levels to concentrations 1000 times higher than those in the external fluid, results in an efficiency carbon fixation reaction in these organisms. These mechanisms are consistent with various results found in the literature about microalgae with high carbon dioxide requirements and capable of accumulating high internal levels of inorganic carbon (Fridlyand et al., 1996; Marcus, 1997; Tchernov et al., 1997; Badger & Price, 2003; Cuaresma et al., 2006).

4. Photobioreactors

Biotechnological processes have been conducted in the evaluation of the mass and energy transference phenomena, in the dimensioning and construction of equipment processing biotransformations and in the operation of control systems and instrument applications for accompanying the transformation kinetics (Merchuk & Wu, 2004).

Photobioreactors using microalgae to treat polluting compounds and produce biomolecules are based on five basic criteria: elevated efficiency in the use of light energy, an adequate mixing system, easy control of the reaction conditions, reduced hydrodynamic stress on the cells and ease in scale-up (Muñoz & Guieysse, 2006).

Systems using photobioreactors are based on natural processes in which the photosynthetic metabolism of the microorganisms converts light energy, heat and CO_2 into photosynthetic

products (Contreras et al., 1999). The use of photobioreactors to cultivate microalgae requires the presence of light, carbon dioxide and dissolved nutrients for growth of the microorganism. Consequently, these processes require systems for illumination, gas exchange (addition of CO_2 and removal of O_2), the addition of nutrients and temperature control (Rorrer & Cheney, 2004).

Photosynthetic microorganisms can be cultivated in open or closed photobioreactors. The closed systems are characterized by elevated photosynthetic efficiency associated with a precise control of the operational variables, showing a lower risk of contamination and minimization of water loss by evaporation, highly significant factors in open systems. On the other hand, closed systems are more expensive, since they must be constructed with transparent materials, and are more complicated to operate and more difficult to scale up. The ratio of volume per unit area is another criterion to be considered when choosing the system, since the implementation of open systems requires the availability of large areas for the elevated reaction volumes (Borowitzka, 1999; Molina Grima et al., 1999).

Various configurations have been proposed for closed photobioreactors. The main types include bubble column, air-lift, tubular (loop) and stirred tank reactors (Jacob-Lopes et al., 2009). Open pond systems can be oval (raceway), circular or rectangular (Borowitzka, 1999).

4.1 Carbon dioxide transfer in photobioreactors

Carbon dioxide is usually the main carbon source in the photosynthetic cultivation of microalgae and can be transferred continually or intermittently from the gas phase to the liquid phase of the culture medium (Molina Grima et al., 1999).

The reactivity of carbon dioxide in aqueous solutions establishes various equilibriums in its contact with water. The first equilibrium refers to the dissolution of the gas in the water, forming carbonic acid. The carbonic acid undergoes almost instantaneous dissociation into bicarbonate and carbonate ions with the total inorganic carbon concentration being given by the sum of the species CO_3^{2-}, HCO_3^- and CO_2 (Rorrer & Mullikin, 1999).

Simple CO_2 bubbling in the liquid phase does not lead to a total dissolution, since a fraction of the injected CO_2 is lost in the gas outlet. CO_2 absorption is mainly a function of the volumetric mass transfer coefficient, the mass transfer driving force and the gas retention time (Merchuk et al., 2000).

In terms of solubility, carbon dioxide is approximately ten times more soluble in water than oxygen gas. Nevertheless, due to the low solubility of both gases in aqueous solution, there is a need to provide these elements throughout the process (Klasson et al., 1991).

Thus an efficient carbon dioxide transfer system is required for photobioreactors. Efficiency in carbon dioxide transfer is necessary so as to raise the volumetric mass transfer coefficients $K_L a$ (CO_2) allowing for improved transfer of gas to the liquid phase (Baquerisse et al., 1999). According to these authors, the volumetric mass transfer coefficients depend mainly on the physical properties of the fluid, the fluid flow and the system and geometry of the gas injector.

Carbon dioxide transfer in bioreactors becomes a limiting factor in the processes, since the dissolved carbon dioxide concentration decreases with increase in temperature and also with an increase in the concentration of dissolved salts. This factor is relevant in processes for the transfer and removal of CO_2 by microalgae, suggesting the need for higher values of saturation concentration (Rorrer & Cheney, 2004).

5. Carbon dioxide biotransformation by microalgae

Microalgae are microorganisms that are being applied in the reduction of carbon dioxide emissions into the atmosphere, where this compound is biotransformed in the presence of light energy. Evidence of a highly developed photosynthetic system has led to the suggestion of using microalgae in the treatment of gaseous effluents with elevated CO_2 concentrations, generated by industrial discharges (Hsueh et al., 2007).

Much research was developed in the nineties, especially in Japan, on processes for the biofixation of carbon dioxide using microalgae. These studies are being intensified, aiming at projecting systems that operate efficiently and economically with the objective of developing technologies for the reduction of gaseous pollutants (Watanabe & Hall, 1996; Watanabe & Saiki, 1997; Cheng et al., 2006; Ono & Cuello, 2007; Jacob-Lopes et al., 2008; Jacob-Lopes et al., 2009, Francisco et al., 2010).

The use of microalgae in carbon dioxide convertion processes is considered a promising alternative, since the element carbon can be converted by different mechanisms. In the first step, the carbon dioxide dissolved in the aqueous phase of the system can be sequestered by chemical precipitation due to the reaction of the ions bicarbonate and carbonate with elements present in the culture medium, such as calcium and magnesium. These reactions are catalyzed by the growth and physiology of the microalgae (Marcus, 1997; Lee et al., 2004). Another carbon-fixing pathway is related to the Calvin-Benson cycle, where specialized enzymes present in these organisms catalyze reactions that incorporate carbon atoms coming from the CO_2 involved in photosynthesis (Falkowski, 1997). The biological conversion of carbon dioxide results in products of the photosynthetic metabolism such as cells, oxygen, biopolymers soluble in the culture medium and volatile organic compounds (VOC's) (Ishida et al., 1997; Muñoz et al., 2004; Jacob-Lopes et al., 2010).

The CO_2 conversion into biomass is high only under conditions where the CO_2 mass loading rate is low. At a high CO_2 mass loading rate, the formation of volatile organic compounds is the main CO_2 biotransformation route (Fig. 1).

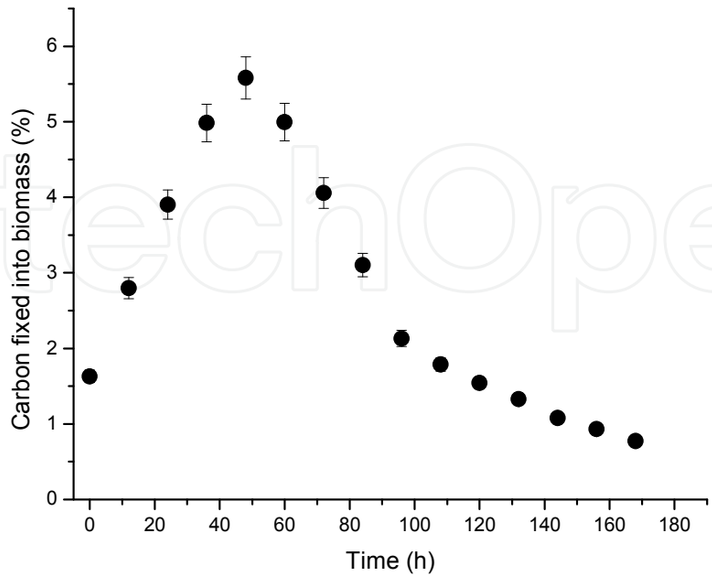

Fig. 1. Percentage of effectively sequestered carbon fixed into biomass. 15% of CO_2 at a flow rate of 1VVM. Source: Jacob-Lopes et al. (2010).

6. Potential uses for the bioproducts

The main advantages of producing biomolecules from photosynthetic organisms are related to their rapid reproduction and the low cost of the sources of energy and nutrients used for their multiplication. Evidently this is done for the cost of the medium in which the microorganisms develop, which can be composed of a wide variety of substrates, some of which, including industrial residues, are cheap, thus solving problems of an environmental nature and also serving to produce consumables (Anupama & Ravindra, 2000).

The biochemical composition of the microalgal cells includes characteristics of commercial interest, significant proportions of proteins, lipids, carbohydrates and pigments, which can be used as ingredients of foods destined for human consumption and animal feeds and in the extraction of biomolecules and the production of biofuels (Harun et al., 2010). In this way, the use of these microorganisms in carbon sequester processes associates the treatment of polluting compounds with the production of consumables that can be recycled in a variety of forms. Table 1 shows some potential uses for the bioproducts formed by the biological conversion of carbon dioxide in photobioreactors.

An analysis of Table 1 demonstrates a wide variety of possible uses for the microalgal biomass. According to Spolaore et al. (2006), the microalgal biomass industry currently produces more than 5000 tons of dried mass/year with an annual revenue greater than US$ 1.25×10^9, not including processed products, demonstrating the exploration potential of this type of biotechnological process.

Application	Examples	Reference
Human food	Source of single-cell protein and use in the supplementation of products such as pastas, soups and beverages.	Rodriguez-Garcia and Guil-Guerrero (2008)
Animal feed	Frequent use of some species in the feeding of fish and shellfish.	Olvera-Novoa et al. (1999)
High-value molecules	Source of chlorophyll *a*, phycocyanin, β-carotene, γ-linolenic acid, eicosapentaenoic acid and stable isotope biochemicals	Spolaore et al. (2006)
Fertilizers	Use of the biomass as a source of nitrogen and phosphorous in tillable land.	Chae et al. (2006)
Natural gas production	Production of CH_4 in fermenters by the digestion of biomass.	Yen and Brune (2007)
Biodiesel production	Production of biodiesel from the lipid fraction of the cells.	Miao and Wu (2006)
Syngas production	Production of synthesis gas from the biomass.	Amin (2009)
Inorganic salts production	Source of carbonates and bicarbonates	Lee et al. (2004)
Renewable production polymers	Source of exocellular sugars and proteins.	Ishida et al. (1997)
Volatile organic compounds production	Production of hydrocarbons, aldehydes and organohalogens	Muñoz et al. (2004)

Table 1. Potential uses of the bioproducts

Besides the use of biomass and its derivatives, carbonates and bicarbonates are other products likely to be formed in photobioreactors. The use of Generally Recognized as Safe (GRAS) species and airstreams without toxic compounds, e.g., bioethanol plants, can produce chemicals of commercial value (Huijgen et al., 2007).
In addition, extracellular proteins and mainly sugars can be secreted into culture media in photobioreactors. Such compounds have several applications in pharmaceutical and food industries, since some may have unique properties for special applications, not found in the polymers currently available. These include use as a bioemulsifier, bioflocculant, agar-agar substitute or cosmetic material as well as other (De Philippis & Vicenzini, 1998).
Finally, microalgae cells can produce non methane hydrocarbon (ethane, ethylene, propane, propylene, butane, isobutane, pentane, hexane, isoprene and ethylene) (Schobert & Elstner, 1980; Shaw et al., 2003), organohalogens (chloroform, trichloroethylene, bromomethane, chloromethane, iodomethane) (Scarratt & Moore, 1996) and aldehydes (propanal, hexanal, n-heptanal, formaldehyde, acetaldehyde, furfural and valeraldehyde) (Schobert & Elstner, 1980; Nuccio et al., 1995). These compounds are continuously being formed and released from the aqueous phase of photobioreactors. The production of renewable polymers is an emergent area for industrial practice.
Thus, microalgae-based systems are one the most promising emerging biorefinery platforms. These systems are a means of resolving environmental problems and providing effective solutions to the energy crisis at the same time. This biorefinery type mediates between environment and society and has positive economic impacts.

7. Applicability of the process

Full-scale processes with microalgae are mainly based on open photobioreactors. Some successful initiatives have been carried out in closed systems, and in this case, the scale is normally semi-pilot or pilot. The intensification of these processes represents an important step in the consolidation of the technology for the biological transformation of carbon dioxide into photosynthetic products.
Open photobioreactors are suitable for the production of high-value products. The carbon dioxide sequestration rates are low and are not viable for processes that aim only to obtain carbon credits.
Closed photobioreactors have higher rates of biotransformation of carbon dioxide into bioproducts, and the greatest potential for commercial application. However, there are still high hurdles to overcome before these processes can be fully scalable.
Although it is believed that there is high availability of industrial CO_2, for use in microalgae-based systems in the practice this is not true. Biologically mediated processes require cold gases, rarely obtained in the conventional industrial flue gases, which can reach thousands of degrees Celsius, limiting the use of most sources of industrial gases.
In addition, flue gases are usually composed of carbon dioxide and water vapor as well as nitrogen and excess oxygen remaining from the intake combustion air. They also contains a significant percentage of other compounds such as particulate matter, carbon monoxide, nitrogen oxides and sulfur oxides, which have a toxic and/or inhibitor effect on microalgal growth.
Finally, there is no consensus on the shape of closed photobioreactors for full-scale application. Bubble column, air-lift, flat-panel and tubular reactors and variations of these are the main options, but they are still far from industrial reality.

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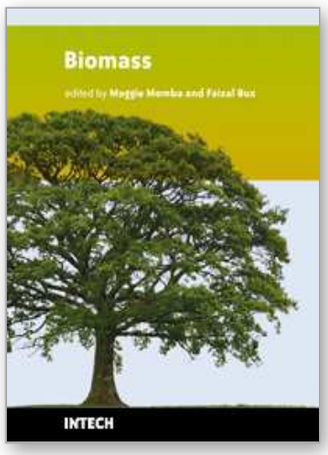

Biomass

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Due to demands placed on natural resources globally and subsequent deterioration of the environment, there is a need to source and develop appropriate technology to satisfy this requirement. For decades mankind has largely depended on natural resources such as fossil fuels to meet the ever increasing energy demands. Realizing the finite nature of these resources, emphasis is now shifting to investigating alternate energy source governed by environmentally friendly principles. The abundance of biomass and associated favorable techno-economics has recently changed global perceptions of harnessing biomass as a valuable resource rather than a waste. To this end this book aims to make a contribution to exploring further this area of biomass research and development in the form of a compilation of chapters and covering areas of ecological status of different types of biomass and the roles they play in ecosystems, current status of biomass utilization and deriving energy and other value added products from biomass. In this context biomass can be defined as large plants and trees and different groups of microorganisms. This book will serve as an invaluable resource for scientists and environmental managers in planning solutions for sustainable development.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
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