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ORIGINAL PAPER

BIOLOGICAL SEQUESTRATION OF CARBON DIOXIDE FROM THERMAL POWER PLANT EMISSIONS, BY ABSORBTION IN MICROALGAL CULTURE MEDIA

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Abstract

The debate on the effects of increased carbon dioxide levels on global climate makes it more difficult for Romania to achieve its target of reducing emissions by 8%, according to the Kyoto Protocol, and the risk management of carbon dioxide emissions becomes one of the priorities in ensuring Romania's economic competitiveness.

Biological carbon sequestration might be the most promising, environmentally friendly and cost-effective means of reducing carbon dioxide emissions in the energy sector. Some selected strains of microalgae act as enhanced natural sinks for carbon dioxide because they are 20 times more efficient than terrestrial plants in utilizing carbon dioxide per square meter of fuel gas.

Our paper aims at providing a selection of microalgae species that are able to convert a significant fraction of carbon dioxide output resulted from power plants into controlled composition biomass by accelerated microalgae photosynthesis. Thirty four strains of microalgae have been isolated and tested on a laboratory scale. The final goal of our research is to obtain biodisel and horticultural oils based on microalgal biomass.

Keywords: Greenhouse Gases (GHG), Carbon dioxide; Microalgae; Biomass; Biodisel;

Introduction

The accumulation of anthropic carbon dioxide in the atmosphere, with all its bad consequences on the human society, is both a reality and a challenge for science and technology nowadays. Certain surveys show that the carbon dioxide level generated by human activities rises up to 20 Gt/year. At the beginning of the industrial era, the carbon dioxide concentration in the air was around 275 ppm; in the first years of the current century the level was considerably higher (367 ppm) and it substantially contributed to the greenhouse effect, which brings long term, uncontrollable, negative consequences on our planet. The warming of the atmosphere is due to the carbon dioxide emissions in a proportion of 52%. In order to avoid reaching a catastrophic, irreversible level of such emissions, the reduction of carbon dioxide emissions in the atmosphere becomes a priority and a proof of responsibility on behalf of the post-industrial human society. The main cause for the increased level of CO_2 in the atmosphere is the use of fossil fuels for the generation of power, transport, industry and home utilities.

The Kyoto Protocol is an amendment to the United Nations Framework Convention on Climate Change (UNFCCC), an international treaty on the global warming due to the greenhouse effect. The states which ratified this Protocol undertook to reduce emissions of carbon dioxide and other five greenhouse effect gases.

Romania was the first state to ratify the Kyoto Protocol, subsequently undertaking a reduction of 8% of the GHG emissions. The recent global reorientation towards coal power plants, generated by the poor supply in hydrocarbons, makes this reduction even more difficult and focuses the research projects in Romania on the reduction of the greenhouse gas (GHG) effect, placing this issue among the top priorities for the achievement of the economic competitiveness.

Surveying the evolution of the GHG emissions in Romania, during the period 1989-2004, shown in figure 1, we can conclude that the carbon dioxide emissions were of 194.826 Gg (acknowledged as a reference value) during the year 1989; in later years, due to the economic recession, the emission level went down to 125,597 Gg in 1994, and to 148,202 Gg (1Gg = 1000 tons) in 2001.

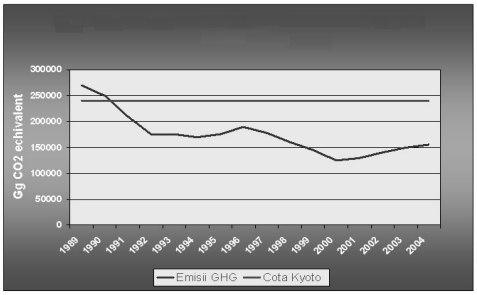

Fig. 1. Evolution of Greenhouse Gas Emissions (GHG) in Romania
(Source: Ministry of the Environment and Water Management (2006)

The estimates on CO_2 emissions for the year 2020 are around 131050 Gg, and the energy sector brings a major impact on the emissions level, up to an approximate percentage of 89%; around 52% of the emissions will result from fossil fuel combustion, and around 24% from the processing industries or constructions.

Many technologies or technological solutions have been advanced in order to reduce the carbon dioxide emissions in the atmosphere. Their general classification is shown in figure 2.

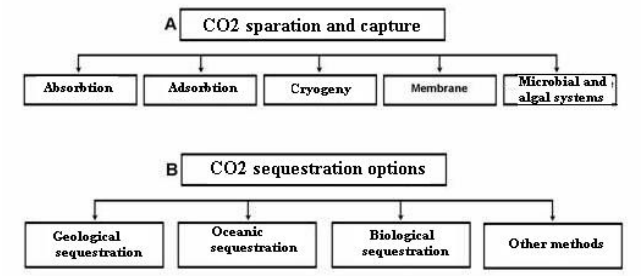

Fig. 2. Proposed solutions for reducing carbon dioxide emissions

Using the photoautotrophic organisms to reduce the noxious effects of industrial CO_2 emissions in the atmosphere by sequestration under the form of biomass constitutes a relatively recent solution. This may become valuable if we obtain high added value products which can become economically and commercially attractive (Kadam, 1997; Watanabe & al, 1997). Due to the price increase in fossil fuel on the international market, this solution may

become more and more feasible (Benemann, 1997).

Numerous microalgae have a superior productivity compared to the other terrestrial plants under proper conditions of light, temperature, nutrients, available CO_2 concentration (Binaghi & al, 2003; Behrens, 2005; MacIntyre & al, 2005).

In principle, all these factors can be controlled and can control the quality of the biomass that has derived from photosynthesis. The initiation of technologies based on microalgae is attractive also because they can be applied at different scales and in different locations (Kadam, 1997).

Biologically, the selection of microalgae species that can be utilized for the efficient sequestration of CO_2 from industrial emissions is not at all simple, as they are different in morphology or behaviour, in their growth processes or the intake of the impact of environmental factors.

Conceptually, this solution is based on the oxygenic photosynthesis of the micro algae, the result of which is used to convert the carbon dioxide from industrial emissions into a biomass that can be economically valuable. Implementing this solution means, among other things, to develop the photobioreactor technology, to use the adequate microalgae and to know all the effects that each component of the industrial emissions may have upon the photosynthesis process (Stewart & al, 2005)

Contrary to the more developed plants, the microalgae achieve the photosynthesis in the water, a condition that brings certain particularities, limitations and specific features to the process. Among these, the intake of CO_2 from the environment and its change into a basis for Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), the key enzyme involved in the carbon fixation, represents a factor of major importance for the efficiency of the photosynthetic carbon fixation (Badger & al, 2000)

In water solutions, the main inorganic carbons for photosynthesis are CO_2, CO_3^{2-}, and HCO_3^- (figure 3). All these carbons diffuse relatively slowly and can convert, by a series of hydrating, dehydrating and proton reactions, altered by the pH of the water environment or the nutritive solution (figure 4). Under normal environmental conditions, the hydrating of CO_2 into HCO_3^- is a very slow process compared to the speed in the CO_2 alteration, during photosynthesis. (Badger & al, 1994).

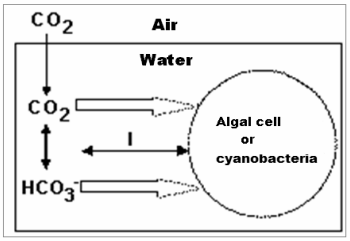

Fig. 3. Inorganic carbon sources for photosynthesis concentration in aquatic

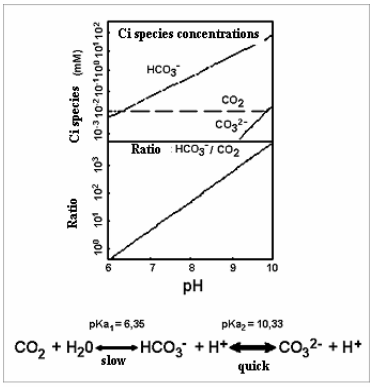

Fig. 4. Distribution of inorganic carbon species in medium. Depending on pH; CO_2 in aquatic medium water is represented being constant (at equilibrium with CO_2 in air)

All aerobic photosynthetic organisms, microalgae included, adapted themselves by developing a CO_2 concentration mechanism (CCM – « CO_2 concentrating mechanism »). The target of this mechanism is to create the proper conditions for an increase in the carboxylation performances of Rubisco (Broda, 1975). Of course, there are differences in CCM, from one group of organisms to the other, but a comparison shall reveal a general similarity. In table 1 the components of this mechanism in microalgae is presented comparatively (Badger & al, 2000).

Table 1. Comparative study between CCM ("CO_2 concentrating mechanism") components in microalgae and cyanobacteria

Nr	**CO_2 concentrating mechanism component**	**Microalgae**	**Cyanobacteria**
1	Active capture of CO_2	CO_2 and HCO_3^- active transport across plasmatic and chloroplasts membrane	CO_2 and HCO_3^- active transport across plasmatic membrane
2	Energy supply during photosynthesis	Active transport energetic supported by ATP produced during photosynthesis	Active transport is supported by ATP and probably NADPH derived from photosynthesis
3	Intermediary specie of CO_2	HCO_3^-, in chloroplast stroma	HCO_3^-, in cytoplasm
4	CO_2 release mechanism	Carbonic anhydrase specifically pyrenoid and thylakoid disks -associated	Carbonic anhydrase specifically localized upon in carboxysome
5	CO_2 concentrating section around Rubisco	Pyrenoid section in chloroplast	Carboxysome section in cytoplasm
6	The loss of CO_2 diminution in CO_2 generation sit by carbonic anhydrase	The special structure of pyrenoid combined with carbonic anhydrase absence in plastid stroma	The special structure of carboxysome combined with carbonic anhydrase absence in the rest of cytoplasm
7	Kinetic properties alteration of Rubisco	Big differences between microalgae groups, especially between green and "non-green" ones. Regarding green microalgae, CO_2 affinity is intermediary and S_{rel} and V_{max} values are increased.	Reduced affinity for CO_2, S_{rel} small values and very high values of $V_{max.}$

The components which are common to all microalgae include: inorganic carbon active transportation systrains into cells, the transportation energy is given by ATP, a photosynthesis product, an inorganic carbon « pool » is formed (HCO_3^-, mainly), which becomes the source of CO_2 for Rubisco, Rubisco falls into different compartments within the carboxysome, or the pyrenoid (with the Eucaryota microalgae) (figure 5).

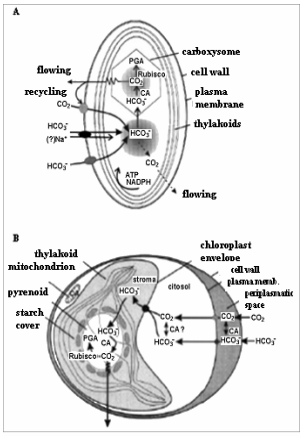

Fig. 5 Cell components identified being CCM-associated in cyanobacteria (A) and eukaryotic microalgae (B)

In these micro-compartments, Rubisco is always accompanied by the carbon anhydrase (figure 5), another enzyme with a major role in the fixation of CO_2, capable to locally generate CO_2 from HCO_3^-. The local association of Rubisco with the carbon anhydrase ensures the immediate intake of the product derived from the activity of carbon anhydrase (CO_2) by Rubisco, and the local rise in the CO_2 concentration, which is essential for the carboxylasic function of Rubisco.

The CCM components in the eukariota microalgae (a specific organizing model for green microalgae is presented) are: several carbon anhydrases, found in the periplasm, chloroplast and cytosol; transporters for the inorganic carbon located both at the level of the plasmatic membrane and the chloroplast envelope; HCO_3^- transporters in the chloroplast stroma; the chloroplast pyrenoid (which contains Rubisco, tilachoids and carbon anhydrase).

In spite of the fact that there is no evidence of a connection between the Rubisco evolution and the proteins involved in light absorption, we can find a definite connection between Rubisco (of different types) and the CCM evolution, the kinetic properties of these forms being complementary to the development of the system components (Delwiche & al, 1996], Watson &al, 1997). In other words, certain "underperforming" kinetic properties of the enzyme are compensated by CCM. Under these circumstances we can speak of a co-evolution of Rubisco and of the CCM system.

From the point of view of utilizing the microalgae for the efficient sequestration of CO_2 emissions, this diversity as well as the Rubisco-CCM co-evolution are very important, as they indicate the fact that Rubisco's kinetic properties are not an important selection criterion of the species; the performances of the growth process, developed under specific conditions, among which a high concentration of CO_2, is by far more important.

We may conclude from the above that the majority of the microalgae have developed solutions in time to adapt to limited concentrations of atmospheric CO_2, respectively to relatively low concentrations of species of dissolved inorganic carbon (DIC – "dissolved inorganic carbon") present in the water environment.

The use of microalgae cultures to sequestrate the CO_2 emissions is supposed to follow a different scenario, in which a much higher CO_2 concentration is needed as an essential condition for this application.

A small percentage increase in carbon dioxide during the process of airing is a common practice to obtain a higher rate in the growth of cultures.

If a microalgae strain growing in a photobioreactor is air-ventilated (1liter/minute) and if we allow a concentration of 0.033% CO_2 in the air it becomes obvious that under proper light conditions (proper for the photosynthesis), the phototrophic growth shall be limited due to the low concentration of carbon dioxide. Considering that all the carbon dioxide is fully accepted into the biomass, having a carbon content of 50%, we can obtain a productivity of 3.54×10^{-4} g biomass/minute. This value is very small.

Productivity improves if we increase the CO_2 (0.2-5%) in the gas flow; this level is recommended by the majority of authors (Lee & al, 1984; Merchuk & al, 2000; Morita & al, 2001; Babcock & al, 2002; Behrens, 2005).

Microalgae react in different ways to the concentration levels of carbon dioxide in the breeding cultures (Miyachi & al, 2003). Certain microalgae are characterized by a growth in the photosynthesis when they are transferred to breeding conditions that include limited CO_2, i.e. by ventilating the cultures with non-enriched air (fig. 6A).

If the activity of the carbon anhydrase is specifically inhibited, the photosynthesis speed is modified (fig. 6B). Other species (for example *Chlorella*) are capable to develop high rates of photosynthesis (a small percentage of CO_2) which they can maintain under carbon

dioxide saturation, but they cannot fix the CO_2 efficiently (fig. 6C). Recently, the scientists noticed that certain species can grow rapidly under conditions of high CO_2 concentrations (over 40%) maintaining high rates of photosynthesis (figure 6D).

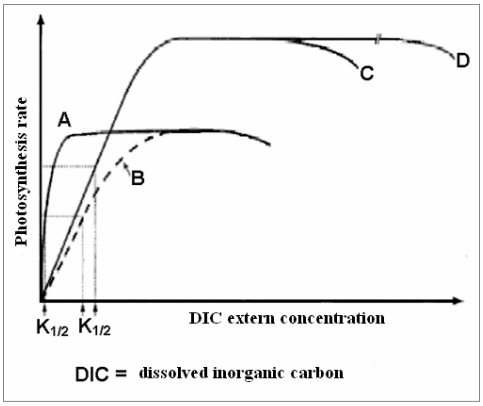

Fig. 6. The relationship between photosynthesis speed and extern concentration of DIC

Generally, the microalgae that can grow under small CO_2 concentrations have a carbonic anhydrase activity 10-20 times higher than the one developed by the microalgae which grow under higher CO_2 concentrations (Miyachi & al, 2003[]).

A similar study was performed on the *Chlorococcum littorale*, a micro alga originating in the sea (Kurano & al, 1995[]). The cells of this species can grow without a *lag* period if they are transferred from 5% CO_2 to 20% and then to 40% CO_2. The inhibition of both the growth and the photosynthesis of the *C. littorale* stem, appears only if the cells are transferred from strains that grow in direct air flow to strains that have a 40% concentration of CO_2, without accommodation.

In this case the inhibition is due to the decrease in the intra-cellular pH, resulted from the carbon anhydrase intra-cellular activity under conditions of excessive CO_2 (Satoh & al, 2001; Berner, 1993; Badger, 1987).

The aim of this study was to select the species and the microalgae strains, from the ones already existing in the Algae Collection of the Biological Research Institute in Cluj-Napoca (AICB), based on the growth potential noticed in standard „batch" cultures, and to use them for the bio-fixation of CO_2 by controlled microalgal photosynthesis, obtaining controlled structures with a high contents of fat in the algal biomass.

Materials and Methods

The experiments were made on a number of 35 microalgae strains living in lakes or rivers and available in the AICB collection (Dragoş & al, 1997) (table 2).

Table 2 List of microalgae strains utilized for selection

Nr	Strain	Taxon name	Exponential growth rate (unit. OD/day)	Doubling time (days)	Cells length (µm)
1	AICB 25	*Chlorella fusca* Shihira et Krauss	0.088	11.33	5.25-(7.08)-8.81
2	AICB 166	*Chlorella fusca* Shihira et Krauss	0.253	3.95	5.23-(7.2)-9.99
3	AICB 170	*Chlorella fusca* Shihira et Krauss	0.120	8.31	4.66-(7.54)-13.12
4	AICB 272	*Chlorella fusca* Shihira et Krauss	0.213	4.70	5.92-(10.51)-14.74
5	AICB 292	*Chlorella fusca* Shihira et Krauss	0.262	3.81	7.2-(10.21)-17.26
6	AICB 425	*Chlorella fusca* Shihira et Krauss	0.138	7.27	4.88-(6.92)-12.51
7	AICB 638	*Chlorella fusca* Shihira et Krauss	0.114	8.79	6.61-(11.13)-15.92
8	AICB 424	*Chlorella homosphaera* Skuja	0.278	3.59	2.9-(4.07)-6.29
9	AICB 431	*Chlorella kessleri* Fott et Novakova	0.143	7.01	4.33-(6.71)-10.44
10	AICB 427	*Chlorella lobophora* Andreeva	0.122	8.17	3.26-(5.56)-7.25
11	AICB 307	*Chlorella luteoviridis* Chodat	0.134	7.45	3.06-(4.18)-7.69
12	AICB 570	*Chlorella luteoviridis* Chodat	0.229	4.36	4.26-(7.17)-13.01
13	AICB 396	*Chlorella saccharophila* (Kruger) Migula	0.151	6.62	3.03-(5.13)-7.92
14	AICB 27	*Chlorella vulgaris* Beijerinck	0.049	20.32	3.06-(4.93)-8.22
15	AICB 58	*Chlorella vulgaris* Beijerinck var. *autotrophica* Shihira et Krauss	0.065	15.38	7.9-(12.36)-19.07
16	AICB 103	*Chlorella vulgaris* Beijerinck	0.116	8.59	3.41-(4.74)-7.25
17	AICB 104	*Chlorella vulgaris* var. *vulgaris* Beijernick f. *viride* Chodat	0.131	7.62	3.37-(4.54)-5.96
18	AICB 311	*Chlorella vulgaris* Beijerinck	0.083	12.08	4.13-(5.7)-8.42
19	AICB 329	*Chlorella vulgaris* Beijerinck	0.117	8.53	5.84-(7.86)-12.7
20	AICB 555	*Chlorella vulgaris* Beijerinck	0.066	15.04	5.18-(8.44)-28.23
21	AICB 565	*Chlorella* sp.	0.151	6.61	4.95-(7.11)-9.22
22	AICB 57	*Coelastrum astroideum* De Notaris	0.060	16.62	4.19-(8.09)-13.32
23	AICB 639	*Coelastrum astroideum* De Notaris	0.169	5.92	7.51-(11.58)-16.35
24	AICB 751	*Coelastrum astroideum* De Notaris	0.063	15.83	6.67-(12.3)-34.12
25	AICB 773	*Coelastrum* sp.	0.085	11.72	5.38-(8.49)-13.52
26	AICB 818	*Coelastrum* sp.	0.121	8.26	5.7-(11.07)-18.69
27	AICB 15	*Chlorobotrys simplex*	0.112	7.97	5.12-(10.24)-14.43
28	AICB 152	*Chlorococcum hypnosporum* Starr	0.086	11.65	9.86-(15.16)-30.67
29	AICB 480	*Chlorococcum hypnosporum* Starr	0.193	5.19	6.7-(11.55)-18.09
30	AICB 408	*Chlorococcum infusionum* (Schrank) Meneghini in Schosser	0.093	10.80	4.72-(11.25)-26.31
31	AICB 43	*Chlorococcum minutum* Starr	0.109	9.18	4.24-(6.98)-11.71
32	AICB 579	*Chlorococcum robustum* (Gartner) Ettl	0.130	7.70	5-(10.54)-28.1
33	AICB 160	*Choricystis guttula* Hindak	0.094	10.63	3.63-(4.7)-6.36
34	AICB 125	*Monoraphidium griffithii* (Berkeley) Komarkova-Legnerova	0.085	11.76	18.41+(26.86)-38.14
35	AICB 141	*Scenedesmus opoliensis* P. Richter	0.061	16.47	15.96-(24.76)-37.04

The cultures were achieved in a Z (Zarrouk) growth medium or a BBM (Basal Bold Medium), recommended for the green algae (Dragoş & al, 1997). The composition of the nutritive Z medium is the one shown in table 3.

Table 3. Composition of Z (Zarrouk) nutritive medium and composition of trace metal stock solution

Components	Conc. (g/l)
$NaHCO_3$	16.80
K_2HPO_4	0.50
$NaNO_3$	2.50
K_2SO_4	1.00
NaCl	1.00
$MgSO_4 \cdot 7H_2O$	0.20
$CaCl_2 \cdot 2H_2O$	0.04
Trace metal solution	1 ml
Chelated iron solution	5 ml
Stock solution components	**Conc. (g/l)**
H_3BO_3	2.860
$MnSO_4 \cdot 4H_2O$	2.030
$ZnSO_4 \cdot 7H_2O$	0.222
MoO_3 (85%)	0.018
$Cu\ SO_4 \cdot 5H_2O$	0.079
$Co(NO_3)_2 \cdot 6H_2O$	0.494

Note: Preparation of chelated iron stock solution: dissolve o.69g $FeSO_4 \cdot 7H_2O$ and 0.93g Na_2EDTA in 80 mL of distillate water by boiling for a short time. After cooling to room temperature solution is made up to 100 mL.

The micro-elements are added from a stock solution, 1 ml for l medium. To the final medium another 5 mL of chelating Fe solution is added. Ingredients are rendered soluble in distilled water.

The composition of the BBM nutritive medium and of the stock solution of micro-elements is shown in table 4.

Table 4. Composition of the BBM nutritive medium and composition of trace metal stock solution

Components	Conc. (g/l)
$NaNO_3$	0.250
KH_2PO_4	0.175
K_2HPO_4	0.075
$MgSO_4 \cdot 7H_2O$	0.075
$CaCl_2 \cdot 2H_2O$	0.025
NaCl	0.025
KOH 85%	0.031
Trace metal solution	1 ml
Chelated iron solution	1 ml
Components – stock solution	**Conc. (g/l)**
H_3BO_3	2.860
$MnSO_4 \cdot 4H_2O$	2.030
$ZnSO_4 \cdot 7H2O$	0.222
MO_3 (85%)	0.018
$CuSO_4 \cdot 5H_2O$	0.079
$Co(NO_3)2 \cdot 6H_2O$	0.494

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For the selection of microalgae strains we used volumes of 15 mL and the cultures were incubated for 12-14 days at a temperature of 20±1°C, under continuous white fluorescent light (approx. 2500 lux; radiation 630 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The growth was determined quantitatively by daily measurements of optical density (OD; spectre-photometry at 678 nm). The growth curves were drawn based on $\Delta \log_2$ OD (deduction of the initial OD out of each

later OD value). The logarithm to the base 2 was preferred, as it allows a rapid calculation of the multiplying/doubling time (= generation time).

For the calculation of the exponential increase rate and of the doubling time we used the protocol described by Wood & al, 2005).

Based on experimental studies, we selected three microalgae strains: *Chlorella homosphaera* - AICB 424 (nutritive Zarrouk medium); *Scenedesmus opoliensis* - AICB 141 (nutritive BBM medium enriched with $NaHCO_3$); *Chlorobotrys simplex* – AICB 15 (nutritive BBM medium enriched with $NaHCO_3$).

In order to establish the best growth parameters, the selected strains were cultivated in a photobioreactor type PBR 25S, a prototype produced by the German company Sartorius (fig. 7).

Fig. 7. Photobioreactor PBR 25 S for microalgae culture

The operational technical characteristics of the photobioreactor are the following: sterilized modular system with a 6 meter long photosynthesis module; transparent material (glass) ; capacity: 3 litres; Light system with fluorescent lamps of 18 W, with a control connection and power supply; sterilized pH control system; temperature control device (0-150^oC), which can be sterilized; pO_2 control device, which can be sterilized; turbidimetric control, which can be sterilized; mixing system for at least 2 gases, with a pressure valve, rotametre and safety valve, which controls the CO_2 admission, depending on the pH; air ventilating system equipped with a filter that can be sterilized, hypodermic needle and pressure stabilizing valve; a 0-40^oC thermostat; peristaltic reversible pump for the medium admission/sampling/recirculation; medium recirculation at a flow of 50 – 5000mL/min; recirculation speed: minimum 15 m/min ; lighting surface: min 4000 cm^2; light flux/lamp: 1200 lm; the light impact – active radiation for the photosynthesis: 5 – 480 μL/ m^2s;

The operation of the photobioreactor:

1) "batch" system

After the inoculation, the photobioreactor operates a number of days under an influx of CO_2, according to the software settings, until the end of the exponential growth stage, when the growth curve becomes linear. After this stage, the suspension enters a decline stage, which is relatively short and then a stationary stage (in which biomass is no longer accumulated).

2) Turbidostat semi-continuous system

When the biomass suspension is at the end of the exponential stage, the cell density is high but the cell multiplication is active (at its peak). At this point, indicated by this photobioreactor by measuring the turbidity (OD – optical density), the photobioreactor is commuted from the "batch" system to the turbidostat system. Within the turbidostat system, the exponential growth rate is equal to the dilution rate, that is the medium inflow = the suspension outflow during a given time span.

In the "batch" system, the microalgal biomass suspension is harvested at the end of the exponential growth period. In the semi-continuous turbidostat system, the "output"

suspension is harvested daily in a quantity of approx 300 mL and processed by centrifugation at a speed of 3700 rpm, for 10 minutes, at room temperature, cleaned with distilled water to wash away the persistent salts in the medium and re-centrifuged in order to obtain a humid biomass.

The algal biomass is processed to extract, characterize and utilize the algal oil as a primary resource for biodiesel, horticulture oils and other by-products of high added value.

Results And Discussions

35 microalgae strains have been studied, most of them belonging taxonomically to the phylum of green algae (*Chlorophyta*), order of *Chlorococcales;* only the *Chlorobotrys simplex* AICB 15 belongs to the phylum of golden algae (*Xanthophyta).*

Most of the green algae belong to the *Chlorella* Genus (21 strains), known for its high growth potential, in several of its species, and for its efficient biomass applications (Watanabe & al, 1997), as commercial nutritional supplements included. The other 13 strains, also chlorococcales, belong to other kin genus: *Coelastrum* (5 strains), *Chlorococcum* (5 strains), *Choricystis* (one strain), *Monoraphidium* (one strain), *Scenedesmus* (one strain) and *Chlorobotrys* (one strain).(table 2). Morphologically, all strains are considered to be unicellular, but the species of *Chlorella* and *Chlorococcum*, *Choricystis* and *Monoraphidium* are represented by singular cells, while the strains of *Coelastrum* and *Scenedesmus* form up characteristic colonies (known under the name of cenobya). The shapes of cells, their average dimensions, as well as their extreme variations (useful data in elaborating the biomass harvesting technology) are presented in table 2.

The growth curves (on the left – the "batch" culture, unstirred, nutritive solution Z/BBM, no carbon dioxide supplement) are exemplified for 3 microalgal strains and the optical microscopy images (on the right –Nikon optic microscope, lens 100x Nomarski, bara=20 μm) are presented in figures: 8, 9, 10. The curves represent the calculated polynomial trend, and the straight lines the standard deviations of the media.

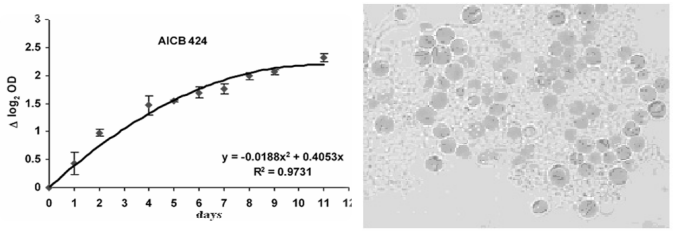

Fig.8. *Chlorella homosphaera* Skuja strain AICB 424

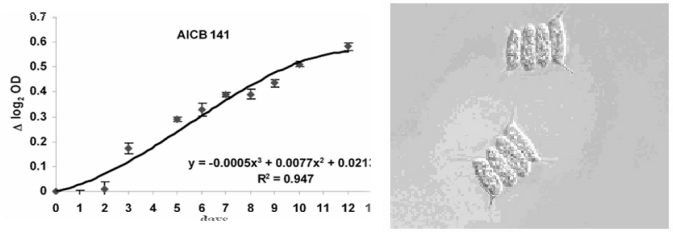

Fig. 9 *Scenedesmus opoliensis* P. Richter strain AICB 141

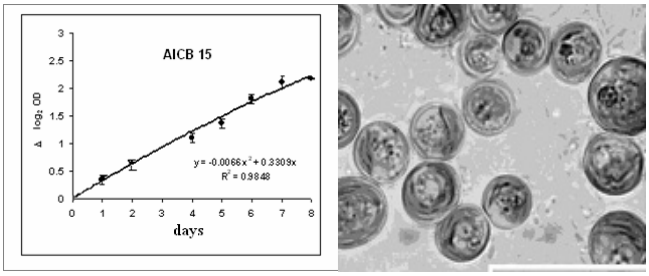

Fig. 10 *Chlorobotrys simplex* AICB 15

The selected microalgae strains were also, cultivated in the photobioreactor and the growth parameters were established in order to obtain controlled structures with the highest possible lipid content. We modified the nutrient BBM medium by enriching with $NaHCO_3$ up to 3-5 g/l, in order to improve the capture and sequestration of CO_2 . The growth diagrams are presented in figures 11, 12, 13.

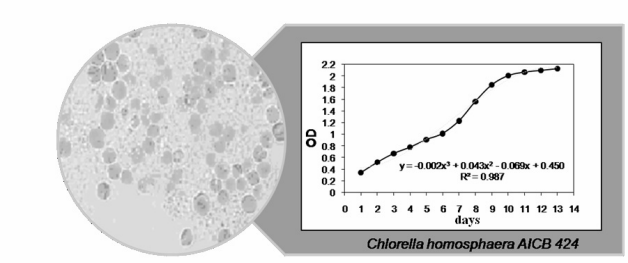

Fig. 11. Diagram of *Chlorella homosphaera* AICB 424 (OD – optical density) growth in Photobioreactor

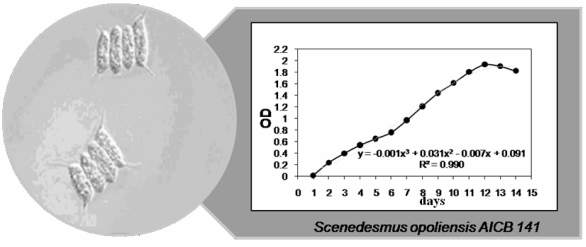

Fig. 12. Diagram of *Scenedesmus opoliensis* AICB 141(OD – optical density) growth in photobioreactor

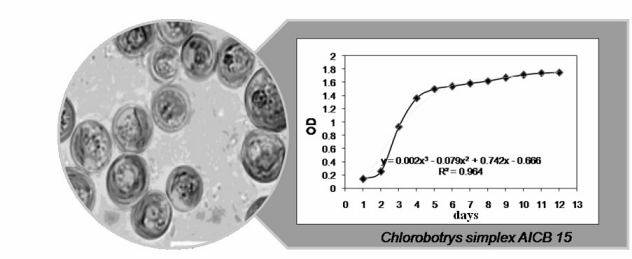

Fig. 13. Diagram of *Chlorobotrys simplex* AICB 15 (OD – optical density) growth in photobioreactor

The efficiency of microalgal cultures in CO_2 biofixation is based on an experimental model, starting from the following premises:

a) Growth process is unrestricted, respectively the parameters of microalgal cultivation (nutrients, light, temperature, optical density, etc) do not limit in any way the process;

b) Chemical specificity of growth medium must be favourable for CO_2 sequestration in the form of bicarbonate ion;

c) Strains specificity for CO_2 capture undertakes to producing biomass during the growth process;

Based on experimental data obtained in photobioreactor (optical density OD, dry biomass, analysis of HCO_3^- in nutrient medium) we calculated a lot of parameters according to Wood and al. (2005) şi Lee & Shen (2004) as shown in table 5. For example, *Chlorella sp* developed an exponential rate about 1.41 day^1(doubling time 0.71 days) and conversion rate of inorganic carbon was about 2g HCO_3^-/g dry biomass/day, respectively 1,5 g CO_2/g dry biomass/day. In other words, 1 kg of *Chlorella* algal biomass (expressed as dry substance) spread into an active suspension, where the growth process is unrestricted, converted 1.5 kg CO_2/day into organic substance.

Table 5 Parameters of CO_2 conversion efficiency into biomass for *Chlorella sp*

Parameter		Value	Std. dev.	Parameter		Value	Std. dev.
Exponential growth rate (day^{-1})	Based on OD	1,41	± 0,06	**Biomass yield vs substratum**	g dry biomass /100 g HCO_3^-	34,61	± 1,50
	Based on dry biomass	1,39	±0,002		g dry biomass./ 100 g CO_2	47,99	± 2,10
Doubling time (days)	Based on OD	0,71	± 0,03		g dry biomass./100 g inorganic C	175,96	± 7,70
	Based on dry biomass	0,72	± 0,01	**Conversion rate of inorganic C into biomass**	g HCO_3^-/g dry biomass./day	2,08	± 0,01
Specific consumption	g HCO_3^-/g dry biomass	2,90	± 0,13		g CO_2/g dry biomass./day	1,50	± 0,10
	g CO_2/g dry biomass	2,09	± 0,09				
	g C anorg./g dry biomass	0,57	± 0,01				

Similarly, we determined that *Scenedesmus sp* developed an exponential rate about 0.87 day^1 (doubling time 1.15 days) and *Chlorobotrys sp* respectively about 1.12 day^1 (doubling time 0.89 days) with $NaHCO_3$ enriched in nutrient medium. In these cases the conversion rate of CO_2 would around 0.9 kg CO_2/day into organic substance for *Scenedesmus sp,* respectively 1.2 kg CO_2/day into organic substance for *Chlorobotrys sp.*

We developed the operation parameters of microalgae growing for performing CO_2 accelerated photosynthesis, in order to obtain controlled composition biomass, with guided properties. The resulted algal biomass was analyzed by thermo-gravimetric methods (TGA) in order to establish its content within the main organic constituents, namely: proteins, lipids, carbohydrates, etc. Figures 14, 15, 16, contain the thermo-gravimetric analyses performed on the algal biomass of *Chlorella, Scenedesmus and Chlorobotrys.*

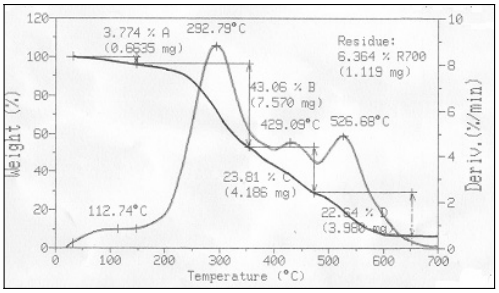

Fig. 14 Thermo-gravimetric analysis of *Chlorella homosphaera* AICB 424 biomass

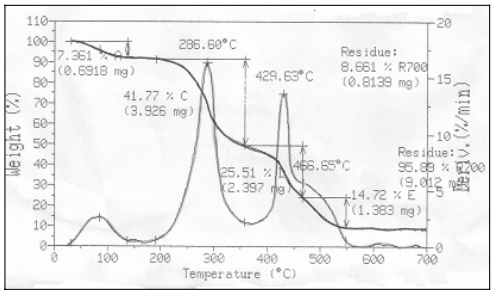

Fig. 15 Thermo-gravimetric analysis of *Scenedesmus opoliensis* AICB 141 biomass

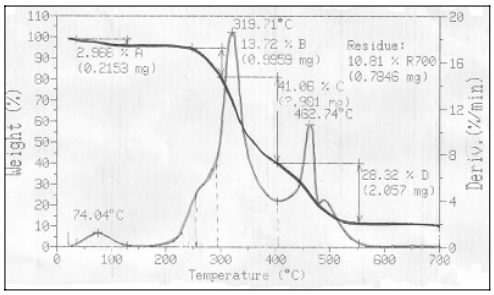

Fig. 16 Thermo-gravimetric analysis of *Chlorobotrys simplex* AICB 15 biomass

Harvested microalgae were shown to consist of 41-43% protein content and 26% algal oil content for *Chlorella sp,* respectively 40% for *Scenedesmus sp* and up to 70% for *Chlorobotrys sp* and carbohydrates by difference, using TGA method. The results are in line with Christi (2007) and Becker (1994) but we succeeded to enhance the content of lipids up to 70% for *Chlorobotrys sp*, cultivated in sparged photobioreactor, with enriched $NaHCO_3$ in nutrient medium.

The algal oils resulted by extraction from the biomass of C*hlorella sp, Scenedesmus sp* and *Chlorobotrys sp* were transesterified, in order to obtain the biodiesel and horticulture oils and were analyzed by gas chromatography (figures 17, 18, 19), in order to determine the distribution of fatty acids (table 6).

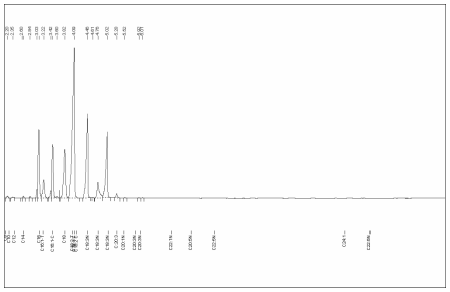

Fig. 17 Gas-chromatographic analysis of *Chlorella homosphaera* AICB 424 algal oil

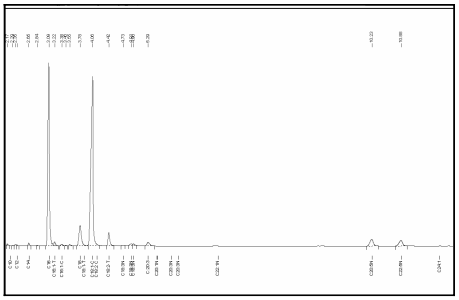

Fig. 18 Gas-chromatographic analysis of *Scenedesmus opoliensis* AICB 14 algal oil

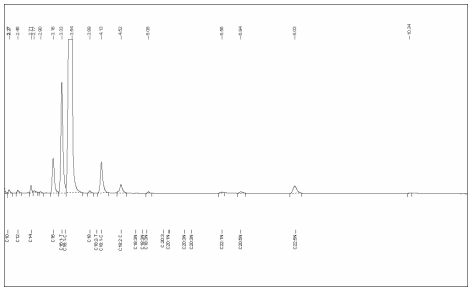

Fig. 19 Gas-chromatographic analysis of *Chlorobotrys simplex* AICB 15 algal oil

Table 6. Distribution of fatty acids in biofuels and horticultural oils obtained from algal biomass by GC

Components	*Chlorella sp*	*Scenedesmus sp*	*Chlorobotrys sp*
C 8	-	-	1.35
C 10	-	-	1.23
C 12	-	-	0.88
C 14	-	-	2.08
C 16	10.64	35.71	15.57
C16:1trans	3.54	0.34	48.99
C16:1 cis	8.20	-	-
C18:0	10.42	6.32	1.08
C18:1 trans	-	-	-
C18:1 cis	0	45.42	15.88
C18:2 cis	35.47	-	4.83
C18:2 trans	-	2.97	-
C18:3n6	14.75	0.08	-
C18:3n3	3.77	0.35	-
C18:3n4	11.97	0.32	0.63

Related to the distribution of fatty methyl esters in biodiesel and horticultural it was found to be predominant C16:0; C16:1cis ; C18:2cis; C18:3n6; C18:3n3; C18:3n4 for *Chlorella sp*, respectively C16:0; C18:0; C18:1cis; C18;trans for *Scenedesmus sp* and C16:0; C16:1trans; C18:1cis; C18:2cis for *Chlorobotrys sp;*

Conclusions

The carbon biological sequestration, in particular the use of technologically adequate photosynthetic strains may be one of the most promising methods for the reduction of the CO_2 emissions in the energy sector, both from the cost and from the environment points of view.

Using the accelerated photosynthesis of the selected microalgae strains we demonstrated that they operate as an intensive natural reducing agent of CO_2 in the flue gas produced by fossil coal power plants and they produce, by cell biosyntheses, lipids, as an alternative primary source for biodisel and horticulture oils, carbohydrates, proteins, carotenoids and other compounds that can become high added value final products.

The paper presents some of the advantages brought by the microalgal culture in the sustainable CO_2 capture and sequestration from industrial emissions

The optimal cell growth, higher photosynthetic efficiency, larger biomass, and algal oil production based on investigation in controlled photobioreactor concluded that *Chlorella sp, Scenedesmus sp and Chlorobotrys sp* could contribute to the creation of a system to produce biofuel and horticultural oils.

Microalgae represent the most important CO_2 consumer; from 1.5 kg of CO_2 sequestrated during the controlled photosynthetic process, we could obtain 1 kg /day of *Chlorella* algal biomass.

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