ACTA MICROBIOLOGICA BULGARICA

A Complex Theoretical Approach for Algal Medium Optimization for CO_2 Fixation from Flue Gas

Alexander D. Kroumov[1]*, Aparecido Nivaldo Módenes [2], Daniela Estelita Goes Trigueros[2]

[1]Department of Applied Microbiology, Institute of Microbiology "Stephan Angeloff"- Bulgarian Academy of Sciences, Acad. G. Bonchev str., Bl.26 , Sofia 1113, Bulgaria
[2]Department of Chemical Engineering - Postgraduate Program, West Parana State University, Campus of Toledo, Rua da Faculdade 645, Jd. La Salle, 85903-000 Toledo, PR, Brazil

Abstract

An robust algorithm of medium optimization for culturing algae was developed. The algorithm takes into account elemental composition of algae, and chemical composition of non-organic source of carbon (in our case, flue gas). Values of the chemical elements in the medium is achieved in linear programming procedure. Medium composition may change on the base of the chosen objective function, for example biomass maximization or overproduction of desired metabolite. The algorithm was checked by performing real experiments with *Chlorella vulgaris* and *Scenedesmus* species in our previous studies (in USA). The results have shown a very good algae growth, when the medium was optimized by using such algorithm. The developed robust algorithm for medium optimization is under continuous verification (in Bulgaria and Brazil) on different algae cultivation processes. The algorithm can be successfully used to design a medium for any bacterial and algae cells with known or unknown physiological requirements for macro- and micro-elements.

Key words: medium optimization, linear programming, algae, flue gas, elemental composition of cells.

Резюме

Разработен е устойчив алгоритъм за оптимизация на хранителна среда за култивиране на водорасли. Алгоритъмът отчита елементния състав на водораслите и химичния състав на неорганичния източник на въглерод (в нашия случай, димни газове). Стойностите на химичните елементи в средата се изчисляват с помощта на метода на линейно програмиране. Съставът на хранителната среда може да се променя на базата на избрания критерий за оптимизация, за максимизиране например биомаса или свръхпродукция на желания метаболит. Алгоритъмът е проверен чрез извършване на реални експерименти с щамове на *Chlorella vulgaris* и *Scenedesmus* в предишни наши изследвания (в САЩ). Получените резултатите показват много добър растеж на водораслите, когато средата се оптимизира чрез използване на този алгоритъм. Проверката на разработения устойчив алгоритъм за оптимизация на хранителна среда се извършва непрекъснато (в България и Бразилия) върху различни процеси на култивиране на водорасли. Алгоритъмът може успешно да се използва за изчисляване на компонентите на хранителната среда за всякакви бактерии и водораслови клетки с известни или неизвестни физиологични изисквания за макро - и микроелементи.

Introduction

Medium optimization for algae culturing is a key point in overall process development. As a common practice, medium optimization for biomass and high value products is done by using statistical experimental design methods (Kathiresan *et al.,* 2007) or simple try and error procedure. Because CO_2 represents a considerable part of the operational costs in mass production of algae (Kadam, 1997) a flue gas can be considered as an excellent choice. In order to reduce the transportation costs, a flue gas from a local coal-fired plant must be used as a source of carbon dioxide for culturing photosynthetic algae. From another point of view, the cultivation of microalgae can also be taken into account as an additional step in flue gas treatment, where CO_2 concentration in the exhaust flue gas is decreased and SO_2 emissions causing acid rain

***Corresponding author**:
e-mail: adkrumov@gmail.com

can be totally eliminated. Global warming situation accelerates political decisions and many countries directed much attention and financial support on studies of the subject.

Hence, utilization of flue gas from coal-fired power plants (Maeda *et al.,* 1995a; Kadam, 2002), industrial heater burning kerosene (Chae *et al.,* 2006), natural gas-fired boiler (Doucha *et al.*, 2005; Doucha and Livansky, 2006), or a simulated flue gas (Brown, 1996) were performed in attempt to make CO_2 fixation from flue gas (Benemann, 1993) a visible cost-effective industrial scale technology. Special attention in the field must be given to international achievements in Europe, Israel, Japan and USA.

However, these studies do not focus on the complex algorithm and how the first steps in process development (medium optimization) were performed. The studies showed a global solutions and ranges of applicability of this technology without showing any details about optimal values of medium components and how they were obtained. As well, there are no studies giving understanding on interactions between water chemistry-algal physiology-flue gas.

Following this thoughts we tried to build a robust algorithm for medium optimization by applying the principles of system analysis theory (Kaffarov *et al.,* 1979, 1985), modern mathematical methods and available software for calculation of chemical equilibrium and speciation of CO_2 and SO_2 in water in order to understand such interactions. The algorithm was used in a step-by-step procedure of active experimental design by culturing *Scenedesmus* an *Chlorella* species with the goal of optimal inorganic carbon utilization from flue gas emissions. The main idea of this robust method was presented in several meetings of Society of American Engineers in Massachusetts and elsewhere in USA (Crofcheck *et al.*, 2009a, 2009b, 2010, 2012a) and as well as elsewhere (Kroumov, 2013; 2014; Kroumov *et al*., 2014). The experimental verification of this approach can be found in details elsewhere (Crofcheck *et al*., 2012b).

In this work, we are going to highlight the theoretical steps of the algorithm and how this knowledge can be used in other levels of complex photobioreactor (PBR) model development. Experimental verification of the developed algorithm was successfully performed at Biosystem Agricultural Engineering Lab, and CAER Lab, at University of Kentucky under the grant "CO_2 sequestration from flue gas by algae for production of lipids, carbohydrates and proteins".

First, several medium recipes for culturing fresh water *Chlorella* were analyzed by applying knowledge on elemental composition of algae biomass and linear programming calculations.

Secondly, N-source, P-source, and micro-elements composition were changed and varied in order to design the cost-effective medium for closed PBR in outdoor cultivation conditions. The flue gas is rich with metals which must be considered as micro-nutrients. Finally, we tried to simulate the SO_2(aq) influence on the microalgae growth and to understand SO_2(aq) interactions with algae for the

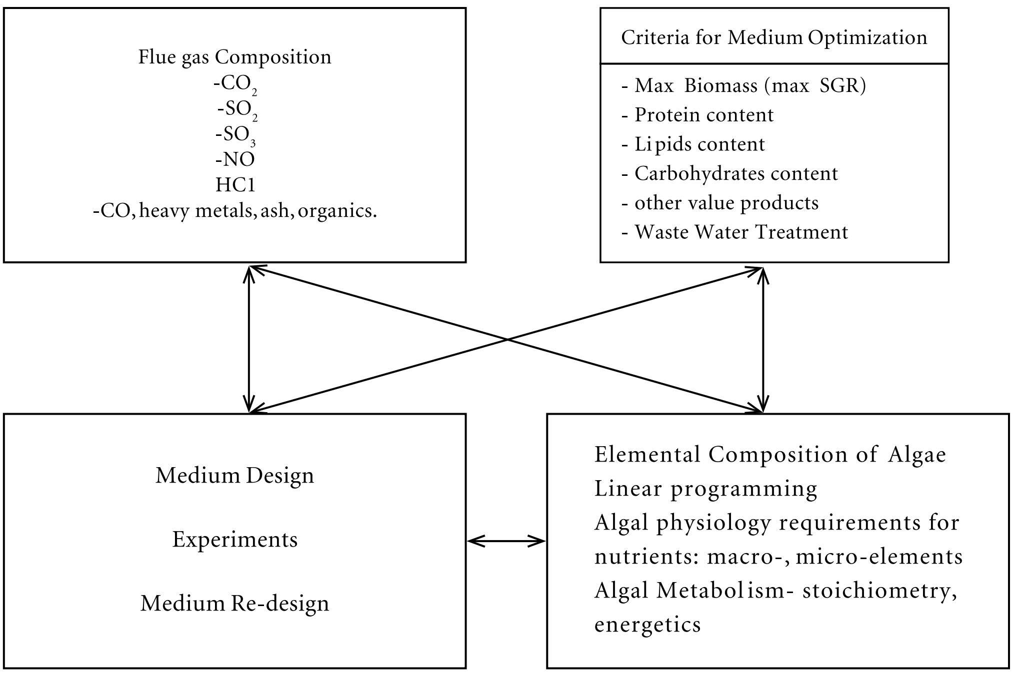

Fig. 1. Graphical representation of the complex theoretical approach for medium optimization

given pH. Water chemistry studies during absorption of flue gas showed that pH may reach values of pH=2.0 which requires buffering the medium or precise pH control. Buffering the medium with sodium bicarbonate as a best choice was studied, as well.

Theory-Development of the Algorithm

Existence of very rich knowledge database on fresh and marine algae physiology and their elemental composition, metabolism, and nutrients requirements for fastest growth opens the space for synthesis of optimal algorithms for medium optimization. Hence, development and application of chemically defined medium is not a single act, it is a procedure which should be focused on practical considerations, especially process economics and the complex interactions of the medium with the environment (absorption of flue gas composition in water, its water chemistry which is linked with the algae physiology). It means interactions between simple chemical components with algae and aqueous species of flue gases (mainly obtained from dissolution of CO_2, O_2, SO_2, SO_3, HCl, NO) must be understood. Development of defined medium and its industrial application should relay on reproducible results. The simplicity of the medium by considering all available resources of macro- and micro- nutrients (from water and flue gas) should be a criterion, as well, in order to permit unlimited long term cultivation. Studying and describing such a system cannot be done in a straightforward procedure and requires decomposition of the system in sub-systems and their analysis and synthesis. Hence, for precise calculation of medium components, an application of software packages (for example, MAPLE) with modern numerical and optimization methods (linear programming) is necessary. Studying water chemistry requires knowledge about thermodynamic properties of chemical components and their possible interactions. MINEQL+4.6 software was used as a powerful tool for studying and evaluating chemical equilibrium aqueous speciation of gases, solid phase saturation states, and precipitation-dissolution.

Combining this knowledge database, we developed an algorithm for medium optimization which is based on the principles of system analysis theory (Fig. 1) (Kaffarov *et al*., 1979, 1985). The sub-systems were determined as follows-I-algae, II-flue gas, III-water chemistry. Understanding of these sub-systems and their interactions pass through formulation of the optimization criterion and "procedure of making of decisions" (medium design-experiments-medium re-design).

I - Algae physiology can be considered as a main sub-system. The theory was based on evaluation of the fresh water medium for culturing *Chlorella* strains. The physiological requirements of fresh water algae can be taken from the elemental composition of the particular strain. Nevertheless, evaluation of the published chemical composition of *Chlorella* is fundamental for algorithm development. The strain and medium selection for industrial application is a loop procedure and here is not going to be discussed.

Algae Biomass Elemental Composition

The mass balance strategy is well known one from chemical engineering practice. Applying it to algal biotechnology is very useful and robust tool when there is no obvious starting point for medium design or selection of available recipes for the given algae strain. We performed the analysis on inorganic medium components which present into the biomass, where the biomass nitrogen content was the reference point.

We assume for simplicity that elemental composition of *Chlorella* sp. does not differ significantly; hence for all calculations, one algae elemental composition was used (where "max" values of chemical elements were chosen.). As first step in medium development, we evaluated the most common medium recipes (such as -N-8 and M-8 (Mandalam, and Palsson, 1988), BG-11(Borowitzka and Borowitzka, 1988), M4N medium (Watanabe and Saiki, 1997), Watanabe Medium (Scragg *et al*., 2002), used in practice for mass culturing of fresh water algae. The criterion of unlimited growth was achieved by exact calculation of the medium components. We applied linear programming technique (coded in MAPLE 12) based on elemental composition of *Chlorella* specie biomass to calculate the exact amount of chemical components and to compare the simulation results with the real recipe values. *Note: Elemental composition values (Min, Max) for N, P, K, Mg, S, Fe, Zn, Cu, Mn were obtained from Oh-Hama and Miyachi, 1988, Chlorella, p. 3-26, in Micro-algal biotechnology, Michael A., and Lesley J. Borowitzka, Cambridge University Press, 1988, p.477.*

For our calculations, the most important 9 inorganic macro-and micro-elements (N, P, K, Mg, S, Fe, Zn, Cu, Mn) were considered. For all simulations, the maximum (Max) values of the chosen algal biomass elements were considered.

Linear Programming-Approach and Application

From the average elemental composition of biological material 9 elements were selected that occur in the highest concentrations. Of course the remaining trace elements can also become depleted, but they are only required in very small quantities, which are supposed already to be present in sufficient amounts as impurities in the used chemicals. A number of inorganic chemical compounds can yield the required elements either as free ions or as small ionic molecules that can readily be absorbed. Development of defined synthetic medium is based on perfect proportion between chemical elements representing stoichiometric elemental composition of biomass. To calculate the required quantities for the various chemicals the method of linear programming, more particularly the algorithm for search of a constrained maximum was used.

The program for linear programming calculations was coded in MAPLE 12 software (property of University of Kentucky, Department of Mathematics). In linear programming, a vector "x" is found that minimizes the quantity c.x subject to the constraints ***m.x > = b*** and x > = 0, in which the vector "x" represents the required quantities of the various chemicals, "c" represents the variable to be minimized (e.g., the number of chemicals to be used or the costs of the chemicals per unit), "b" gives the required concentration in the mixture. The composition of the various chemicals may include one chemical element, which should be considered during the calculations. More flexibility can be achieved when the constraints for each variable can be varied. The algorithm constraints search for and find the global maximum of a function in the domain specified by a number of equations (equalities or strict or non-strict inequalities). It must be noted that algorithm is not restricted to the use of linear programming as a maximum search method. For algal medium design can be used statistical (factorial design) and modern search methods such as - genetic algorithms, artificial neural networks, fuzzy sets and their combinations.

Algae Physiology Requirements for Nutrients

The maximum cell density and specific growth rate of algae as a function of nutrients and environmental conditions are well documented in books (Borowitzka and Borowitzka, 1988; Amos, 2004). This knowledge directed our scientific efforts toward the study of optimal nitrogen sources including fertilizers for *Chlorella* maximum growth. For flue gas applications it is important to evaluate the influence of micro-elements such as V, Mo, Co, Ni and utilization of yeast extract in some applications. From financial point of view, it is challenging to evaluate the *Chlorella* growth response on completely fertilizer medium with combination of wastewaters rich with inorganic nutrients. In this sense, water recycling can be considered as an option, as well but some physiological test on auto-toxins accumulation and influence on overall process performance must be performed.

Algal metabolism interacts with the environment by changing its pH value. It is challenging to determine the ability of algae to synthesize low molecular compounds siderophores (Naito *et al*., 2008) in order to utilize the chemical components of the medium which precipitates (Fe, Ca, Mg ions) under the high pH values pH>8.0 and become bio-unavailable.

Algae Metabolism

General approach for medium design is based on solely empirical data and the cells are considered as a black box system. Of course, achievements in metabolic engineering showed a new direction in medium design strategies. This direction is to evaluate the metabolic pathway needs of the algae cells. Combination of stoichiometric models of intracellular metabolites and the mass balance approach seems to have bright future for the medium design. In the future, detailed metabolic and energy flux analysis is expected to be of tremendous help in medium development procedure. Current approaches of medium design in biotechnology includes utilization of the statistical factorial design methods (Kim *et al.*, 2008; Jeong *et al.,* 2008) and the most modern optimization techniques artificial neural networks (Kennedy, 1992), fuzzy sets (Kennedy *et al.,* 1995), genetic algorithms (Weuster-Botz *et al*., 1995; Zuzek *et al*., 1996) and their combinations, but once again many experiments are required and the cell is considered as a black box. It must be noted, that application of statistical and optimization techniques is very powerful tool for medium design, but they are not "panacea". Their search space must be localized between algal metabolic and financial constrains. Further developments on this subject are expected.

II-Flue Gas as a Sub-System

According to our algorithm of medium design, two important interactions of the flue gas in the liquid phase can be considered.

1. pH changes of the medium during absorption of flue gas.
2. How the flue gases speciation in the liquid phase will affect the algae growth.

Flue gas composition and absorption in aqueous and alkaline solutions is well studied and modeled process in chemical engineering practice (Marocco and Inzoli, 2009; Gomez *et al.*, 2007; Desch, *et al.*, 2006). From these studies is obvious that pH of the non-buffered liquid phase dramatically changed and can reached pH=1-2. Hence, buffering the broth for performing algae non-limiting growth on flue gas is obligatory and NaOH ($NaHCO_3$) is the best choice from chemical (Wylock *et al*, 2008; Chang and Rochelle, 1985), and micro-algal physiology point of view (Hsueh *et al*., 2007). To catch CO_2 from flue gas by chemical reaction to produce carbonates (e.g., Na_2CO_3) and use the latter as the carbon source for micro-algal cultivation is very attractive perspective.

For some algae species, combination of SO_x and NO_x have some toxic influence on the algae growth (Lee *et al*., 2002).

Hence, special interest has been given on algae cultivation process by using flue gas (actual or simulated, where algae tolerance to high CO_2concentration in presence of SO_x and NO_x impurities was extensively studied (Maeda *et al*., 1995b; Yanagi *et al*., 1995; Negoro *et al*., 1991, 1993; Lee *et al*., 2002, Jeong *et al*., 2003).

The flue gas compositions varies from one to another coal-fired plant and its content can be found in the literature. We focused our attention on CO_2 (up to 15%) and SO_2 (up to 700 ppm) content which were crucially important to build the proper and optimal medium.

III Water Chemistry

Analyzing the flue gas composition the influence of SO_x can be considered crucially important. We assume that the problems with ash, organics and heavy metals presented in flue gases have been solved and only gases are entering PBR.

Hence, aqueous phase chemistry of the flue gases must be completely understood in order to be applied effective unit operation for gases absorption and their latter utilization by algae.

Evaluation of flue gas composition showed that we may safely restricted our analysis to the dynamics and instantaneous equilibrium reactions of six dissolved species namely: CO_2(aq), HCO_3^-; CO_3^{2-}, SO_2(aq), HSO_3^-, SO_3^{2-}. Oxidation of SO_3(g) in the gas and liquid phases gives sulfate which is a component of algal medium. All other gases can be considered as no harmful impurities and their interactions with algae and chemical species in the liquid phase will be neglected.

Water Chemistry of Flue Gas (CO_2 and SO_2)

The absorption of flue gas in water and alkaline solutions is well studied physical -chemical process and details can be found elsewhere. For our practical purposes special interest is given to the process of flue gas absorption into bicarbonate/ carbonate solution (Ebrahimi *et al.,* 2003). The authors modeled speciation of CO_2 and SO_2 flue gas components by considering the following chemical reactions

$$CO_{2(aq)} + H_2O \leftrightarrow HCO_3^- + H^+ \cdots (1)$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \cdots (2)$$

$$CO_{2(aq)} + OH^- \leftrightarrow HCO_3^- \cdots (3)$$

$$[C_{Total}] = [CO_{2(aq)}] + [HCO_3^-] + [CO_3^{2-}] \cdots (4)$$

where C_{total}- stands for total dissolved inorganic carbon dioxide concentration.

Water Chemistry of SO_2

Dissociation of SO_2 includes the following reactions

$$\begin{aligned} &SO_{2(g)} + H_2O \leftrightarrow SO_{2(aq)} \leftrightarrow \\ &\leftrightarrow H_2SO_{3(aq)} \leftrightarrow HSO_3^- + H^+ \end{aligned} \cdots (5)$$

$$HSO_3^- \leftrightarrow SO_3^{2-} + H^+ \cdots (6)$$

$$SO_{2(aq)} + OH^- \leftrightarrow HSO_3^- \cdots (7)$$

$$[SO_{Total}] = [SO_{2(aq)}] + [HSO_3^-] + [SO_3^{2-}] \cdots (8)$$

where SO_{total}-stands for total sulfite concentration.

According to them the reaction (2, 5 and 6) are very fast. Hence, instantaneous equilibrium is assumed for the reactions (2), (5) and (6) throughout the liquid phase. The hydrolysis of CO_2 is slow. Two reactions (1) and (3) take place in the absorption process of CO_2 by aqueous alkaline solutions. Details about rate and dissociation constants values and their temperature dependence are cited by the authors accurately. It can be noted that the forward rate constants values of reactions (1) and (3) differ (in orders) as a function of pH values below 8 and above 10. Thus, buffering the algal medium with sodium bicarbonate (as a depot of CO_2) or pH control by NaOH has to be considered.

Procedure of Making of Decisions

"Medium design - Experiments - Medium re-design" we called sub-system where the decisions are made on the base of all available information from other sub-systems for the chosen criterion of optimization. It must be noted that the "pure" water chemistry, "pure" flue gas absorption and speciation, and "pure" algae growth can be evaluated (on the base of literature data) separately without experimental verification. Only, interactions between sub-systems and concurrent hypothesis should be checking out for particular working conditions and algae species.

Criterion for Medium Optimization

Determination of criterion for medium optimization is different in research studies as well as in the studies on industrial applications. Criterion for research studies usually is selected to maximize the algae growth under high CO_2 content in the liquid phase. The medium may contain different sources of growth factors and vitamins supporting maximum growth. On the other hand, CO_2 sequestration in industrial conditions requires minimization of costs; hence the medium must be optimized by taking into account algal physiology and growth of algae on cheapest possible sources of nitrogen, phosphorus etc. It means available wastewater source rich with

Table 1. Evaluation of N-8 and M-8 medium composition on the basis of elemental composition of the biomass and by applying linear programming procedure (LPP)

Medium				
Chemical compound	N-8	LPP results	M-8	LPP results
-	g/L	g/l	g/L	g/L
Macro-elements of the medium				
KNO_3	1.0	1.000	3.0	3.000
KH_2PO_4	0.74	0.158	0.74	0.474
NaHPO4	0.26	0	0.26	0
$CaCl_2.2H_2O$	0.013	0.00528	0.013	0.00158
$FeSO_4.7H_2O$	0.00001	0.0493	0.13	0.148
$MgSO_4.7H_2O$	0.05	0.146	0.4	0.437
Micro-elements of the medium				
	g/L	g/l	g/l	g/L
$MnCl_2.4H_2O$	0.01298	0.000648	0.01298	0.001944
$CuSO_4.5H_2O$	0.00183	0.000283	0.00183	0.000848
$ZnSO_4.7H_2O$	0.0032	0.000396	0.0032	0.00119

inorganic component can be an option.

Designing the medium under different criteria makes possible to prepare catalog of defined synthetic media which will serve in pilot plant studies and further their industrial applications in closed PBR configurations. This approach is predetermined because constraints applied for designing the medium for lab studies and industrial design are different. Most common constraints and challenges encountered in lab studies are as follows: time for medium development; cost of development efforts; lack of shaker space; precipitation reaction; foaming; water quality; dispersion or dissolution of solid medium components; effect of components on assay techniques; effect of components on downstream product purification.

For design of an industrial medium most common encountered constrains are as follows: availability of raw materials throughout the year; batch to batch variability of medium components; transport costs of medium components; medium cost and price fluctuations of medium components; contamination problems; water recovery of used medium.

Following this algorithm, *Chlorella vulgaris UTEX 2714* growth behavior and *Scenedesmus* one was studied in different media and conditions. The experimental verification of the method can be found elsewhere (Crocheck *et al*., 2012).

First Step

As a first step in this algorithm we have focused on evaluation of the media compositions most commonly used for mass culturing of *Chlorella sp*. We chose the maximum values of 9 components (N, P, K, Mg, S, Fe, Zn, Cu, Mn) in order to calculate the defined medium by avoiding any algae growth limitations. Further, we evaluated the most common medium recipes (such as -N-8 and M-8 (Mandalam and Palsson, 1988), BG-11(Borowitzka and Borowitzka, 1988), Watanabe's Medium (Scragg *et al*., 2002) and M4N medium (Watanabe and Saiki, 1997), used in practice for mass culturing of *Chlorella*.

For lab studies in flasks BG-11, M4N, and Watanabe's Medium were eliminated. However, their recipes can be re-designed and applied for different purposes in outdoor big scale, when vigorous mixing and high light intensity conditions during the summer time are common.

Evaluation of N-8 and M-8 medium was done previously by authors (Mandalam and Palsson, 1998). The authors' criterion was biomass maximization (high density culture) and their conclusions were that the N-8 medium is deficient in iron, magnesium, sulfur, and nitrogen at high cell densities and long term cultivation. It should be noted that no difference in biomass and chlorophyll content of *Chlorella vulgaris* was observed during 120 h of cultivation in controlled bioreactor conditions.

By using elemental composition of biomass and linear programming procedure we evaluated in what extend these two medium were stoichiometrically balanced. The results are shown in Table 1. The assumption of performing LPP procedure were as follows: CO_2 in the liquid phase, light availability, and mixing are not limiting factors; N-source is reference for calculation of biomass concentration; sodium ions are not limiting for fresh water algae and are not included in LPP because in bioreactor conditions these ions are supplied by both tap water and titration in controlling pH level.

The software program of LPP was coded in MAPLE 12. The LPP results have shown that one can expect biomass concentration of 1.799 g/L for N-8 medium and 5.398 g/L for M-8 medium, respectively in controlled bioreactor working conditions. Comparing the medium recipes with LLP results the following conclusion can be made:

First, phosphates concentration in both medium is high. Addition of $NaHPO_4$ can be avoided. Calcium concentrations difference is not crucial for real outdoor cultivation of fresh water algae because tap water or industrial water is a source of Ca. Sulfates are not a problem, as well, which is in agreement with water chemistry of flue gases SO_2, SO_3. Magnesium, and iron in N-8 medium are not optimal, which was shown by the authors (Mandalam and Palsson, 1998). In our case, micro-elements of both medium are not a concern because flue gas and technical grade chemical components are their good source.

Hence, the algorithm works well and without performing the experiments we were able to build a kinetic hypothesis and to localize the optimum area of experimental study. Moreover, the very first experiment with *Chlorella vulgaris UTEX 2714* in flasks showed an excellent result.

For preliminary study, we choose M-8 medium for flask experiments to evaluate the growth behavior of 3 algae strains. *Chlorella vulgaris UTEX 2714* and *Chlorella sorokiniana UTEX 1230* were obtained from the culture Collection of Algae at The University of Texas at Austin, USA. A wild "unidentified strain" was isolated from water near the Eastern Kentucky Coal-Fired plant (data are property of U-ty of Kentucky and are not shown). The algorithm works excellent and can be applied for development of any synthetic fresh and sea water medium.

Also, it must be noted that the best medium chosen from flasks experiments not obligatory will be the best medium for large scale cultivation in PBRs. The problem is that during the scale-up procedure, the medium composition is changed to meet the control strategies requirements, for example, fed batch addition of nutrients.

According to our algorithm of medium development emphases can be given on following experiments by using fresh water algae and flue gas as a non-organic carbon source.

1. Experiments with M-8 medium -results and discussion;
2. Experiments with urea -results and discussion;
3. Experiments with different medium components and deionized and tap water- results and discussion;
4. Experiments with micro-nutrients -V, Co, Mo, Ni- results and discussion;
5. Experiments without complexation of Iron by EDTA to study siderophores synthesis ability in algae (Naito et al., 2008)- results and discussion;
6. Experiments -buffering the system-Titration with H_2SO_4 and buffer $NaHCO_3$.
7. Experiments with simulated flue gas (Na_2SO_3)- results and discussion;

Note: Based on the LPP, many important medium with industrial application industrial for culturing algae were evaluated. (For example:BG-11 medium; Zarrouk's Medium; Bold's Basal Medium-BBM; Ogbonna-Tanaka Medium; Modified Ogbonna-Tanaka Medium). We have build a catalog of media recipes and the LPP verification is under way through the Brazilian project "Science without borders", 2014-2017.

Conclusions

This work demonstrated that a complex algorithm for medium optimization for culturing algae (in our case *Chlorella*) can be successfully applied in lab conditions by minimizing money, time and scientific efforts. The chosen strains performed well with urea as a nitrogen source. Microelements were necessary for maximum growth of *Chlorella* and the strain utilized yeast extract as a source of vitamins. The findings well complemented most of the previous reports, which focused mainly on culturing *Chlorella* on flue gas. Analysis of the maximum specific growth rate (SGR) showed high potential of the strain for CO_2 sequestration from flue gas. Fertilizer medium can be safely used as a substitution of M-8 synthetic medium for outdoor mass production.

The main advantage of the algorithm is that avoids excessive unproductive experimentation. Its flexibility allows selecting the strains and medium in optimal way (loop procedure) before scale up to outdoor conditions in order to prepare different scenarios of micro-algal performance and adaptability.

In order to achieve very high productivity of such alga, medium re-design and different mode of controlled operations must be studied in pilot plant scale where light availability, mixing conditions and process control strategies are going to play crucial role.

Acknowledgments

The author is grateful to PIs Czarena Crofchek, and Rodney Andrews who gave me opportunity to work on the "Algae project" at University of Kentucky. Part of this work was done under Bulgarian grant (ДФНИ-Е01/0001). The financial support of Brazilian project of CNPq №313737/2014-2, "Science without borders", 2014-2017 and scholarship for "Special Visiting Researcher" of the author are gratefully acknowledged, because the program gives the opportunity to apply and verify the LPP for many algae cultivation processes.

References

Amos, R. (2004). Handbook of Microalgal culture: Biotechnology and Applied Phycology, Blackwell Science Ltd., p. 566.

Benemann, J. R. (1993). Utilization of carbon dioxide from fossil fuel burning power plants with biological systems. *Energ. Convers. Manage.* **34**: 999-1004.

Borowitzka, M. A., Lesley J. Borowitzka (Eds.) (1988). Micro-algal biotechnology, University Press, Cambridge, pp. 477.

Brown, L. M. (1996) Uptake of carbon dioxide from flue gas by microalgae. *Energ. Convers. Manage.* **37**: 1363-1367.

Chae, S. R., E. J. Hwang, H. S. Shin (2006). Single cell protein production of *Euglena gracilis* and carbon dioxide fixation in an innovative photo-bioreactor. *Bioresour. Technol.* **97**: 322-329.

Chang, Chung-Shlh, G. T. Rochelle (1985). SO_2 Absorption into NaOH and Na_2SO_3 aqueous solutions. *Ind. Eng. Chem. Fund.* **24**: 7-11.

Crofcheck, C., M. Montross, A. Shea, W. Chen, W. Adams, A. Kroumov, R. Andrews, M. Crocker, S. Morton, C. Fisk (2009a). Selection of microalgal strains and medium optimization for CO_2 mitigation from flue gas. ASABE Annual International Meeting in Reno, NV, June 2009.

Crofcheck, C., M. Montross, A. Kroumov, A. Shea, W. Chen, W. Adams, R. Andrews, M. Crocker, S. Morton, C. Fisk, J. Groppo (2009b). Selection of Microalgal Strains and Medium Optimization for CO2 Mitigation from Flue Gas. Annual Institute of Biological Engineering Meeting in Santa Clara, CA, March 2009.

Crofcheck, C., M. Montross, K. Cassidy, A. Kroumov, A. Shea, W. Chen, R. Andrews, M. Crocker, S. Morton, C. Fisk, M. Wilson (2010). Medium Optimization for CO_2 Mitigation from Flue Gas. Annual Institute of Biological Engineering Meeting in Cambridge, MA, March, 2010.

Crofcheck, C., M. Monstross, E. Xinyi, A. Shea, M. Crocker, R. Andrews (2012a). Influence of media composition on the growth rate of *Chlorella vulgaris* and *Scenedesmus acutus* utilized for CO_2 mitigation. ASABE Annual International Meeting, Hilton Anatole, Dallas, Texas, USA, July 29 - August 1, 2012, Paper Number: 12-1337035. p. 18.

Crofcheck, C., E. Xinyi, A. Shea, M. Montross, M. Crocker, R. Andrews (2012b). Influence of media composition on the growth rate of *Chlorella vulgaris* and *Scenedesmus acutus* utilized for CO_2 mitigation. *J. Biochem. Technol.* **4**: 589-594.

Desch, W., K. Horn, G. Propst (2006). Computation of equilibria in models of flue gas washer plants. *Comput. Chem. Eng*. **30**: 1169-1177.

Doucha, J., F. Straka, K. Livansky (2005). Utilization of flue gas for cultivation of microalgae (*Chlorella sp.*) in an outdoor open thin layer photobioreactor. *J. Appl. Phycol.* **17:** 403-412.

Doucha, J., K. Livansky (2006). Productivity, CO_2/O_2 exchange and hydraulics in outdoor open high density microalgal (*Chlorella sp.*) photobioreactors operated in a Middle and Southern European climate. *J. Appl. Phycol.* **18**: 811-826.

Ebrahimi, S., C. Picioreanu, R. Kleerebezem, J. J. Heijnen, M. C. M. van Loosdrecht (2003). Rate-based modelling of SO_2 absorption into aqueous $NaHCO_3/Na_2CO_3$ solutions accompanied by the desorption of CO_2. *Chem. Eng. Sci.* **58**: 3589-3600.

Gomez, A., N. Fueyo, A. Tomas (2007). Detailed modeling of a flue-gas desulfurization plant. *Comput. Chem. Eng.* **31**: 1419-1431.

Hsueh, H. T., H. Chu, S. T. Yu (2007). A batch study on the bio-fixation of carbon dioxide in the absorbed solution from a chemical wet scrubber by hot spring and marine algae. *Chemosphere*. **66**: 878-886.

Jeong, M. Lee, J. M. Gillis, J.-Y. Hwang (2003). Carbon dioxide mitigation by microalgal photosynthesis. B. *Korean Chem. Soc.* **24**: 1763-1766.

Jeong, Sung-Eun, J.-K. Park, J.-D. Kim, In-J. Chang, S-J. Hong, S.-Ho Kang, Ch.-G. Lee (2008). Statistical optimization of the growth factors for *Chaetoceros neogracil*e using fractional factorial design and central composite design. *J. Microbiol. Biotech*. **18**: 1919-1926.

Kadam, K. L. (1997). Plant flue gas as a source of CO_2 for microalgae cultivation. Economic impact of different process options. *Energ. Convers. Manage.* **38**: 505-510.

Kadam, K. L. (2002). Environmental implications of power generation via coal-microalgae co-firing. *Energy* **27**: 905-922.

Kaffarov, V. V., A. J. Vinarov, L. S. Gordeev (1979). Modeling biochemical reactors. Publisher-Lesnaya Promyshlenost, Moscow, pp. 344, (in Russian).

Kaffarov, V. V., A. J. Vinarov, L. S. Gordeev (1985). Modeling and system analysis of biochemical industries. Publisher-Lesnaya Promyshlenost, Moscow, pp. 280. (in Russian).

Kathiresan, S., R. Sarada, S. Bhattacharya, G. A. Ravishankar (2007). Culture media optimization for growth and phycoerythrin production from *Porphyridium purpureum*. *Biotechnol. Bioeng*. **96**: 456-463.

Kennedy, Max J. (1992). Desining fermentation media: A comparison of neural networks to factorial design. *Biotechnol. Tech.* **6**: 293-298.

Kennedy, Max J., N. R. Spooner (1995), Using fuzzy logic to design fermentation medium: A comparison to neural networks and factorial design. *Biotechnol. Tech*. **10**: 47-52.

Kim, S. Jin, H. K. Lee, J. Han Yim (2008). Statistical optimization of medium components for the production of prodigiosin by *Hahella chejuensis KCTC 2396. J. Microbiol. Biotech.* **18:** 1903-1907.

Kroumov, A. D. (2013). A Global Analysis of CO_2 Fixation System on Waste Gases Example Complex Rate Based Modeling of CO_2 and SO_2 speciation in water, 8th Balkan Congress of Microbiology, Veliko Tarnovo 2013.

Kroumov, A. D. (2014). System analysis of CO_2 sequestration from waste gases by microalgae in closed innovative photobioreactor (PBR). Rate based modeling of the PBR system. Examples, 13th Congress of Microbiologists in Bulgaria with International Participation, Tryavna, October 7th - 10th, 2014.

Kroumov, A. D., G. V. Gacheva, P.S. Pilarski (2014). Mathematical analysis and experimental study of photobioreactor design system for CO_2 mitigation from biogas, 13th Congress of Microbiologists in Bulgaria with International Participation Tryavna, October 7th - 10th, 2014.

Lee, Jin -Suk, Deog-Keun Kim, Jun-Pyo Lee, Soon-Chul Park, Jong -Ho Koh, Hye-Sung Cho, Seung-Wook Kim (2002). Effect of SO_2 and NO on growth of *Chlorella sp KR-1. Bioresour. Techno*l. **82**: 1-4.

Maeda, K., M. Owada, N. Kimura, K. Omata, I. Karube (1995a). CO_2 fixation from the flue gas on coal-fired thermal power plant by microalgae. Energy Convers. Manage. **36**: 717-720.

Maeda, K., M. Owada, N. Kimura, K. Omata, I. Karube (1995b). CO_2 fixation from the flue gas on coal-fired thermal power plant by microalgae. *Energ. Convers. Manage.* **36**: 717-720.

Mandalam, R. K., B. Ø. Palsson (1988). Elemental balancing of biomass and medium composition enhances growth capacity in high-density *Chlorella vulgaris* cultures. *Biotechnol. Bioeng*. **59**: 605-611.

Marocco, L., F. Inzoli (2009). Multiphase Euler-Lagrange CFD simulation applied to wet flue gas desulphurization technology. *Int. J. Multiphas. Flow.* **35**: 185-194.

Naito, K., I. Imai, H. Nakahara (2008). Complexation of iron by microbial siderophores and effects of iron chelates on the growth of marine microalgae causing red tides. *Phycol. Res.* **56**: 58-67.

Negoro, M., A. Hamasaki, Y. Ikuta, T. Makita, K. Hirayama, Sh. Suzuki (1993). Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. *Appl. Biochem. Biotech.* **39-40**: 643-653.

Negoro, M., N. Shioji, K. Miyamoto, Y. Miura (1991). Growth of microalgae in high CO_2 gas and effects of SO_x and NO_x. *Appl. Biochem. Biotech*. **28-29**: 877-886.

Scragg, A. H., A.M. Illman, A. Carden, S.W. Shales (2002). Growth of microalgae with increased calorific values in a tubular bioreactor. *Biomass Bioenerg.* **23**: 67-73.

Spaargaren, Dirk H. (1996). The design of culture media based on the elemental composition of biological material. *J. Biotechnol.* **45**: 97-102.

Watanabe, Y., H. Saiki (1997). Development of a photobioreactor incorporating *Chlorella sp*. for removal of CO_2 in stack gas. *Energ. Convers. Manage.* **38**: 499-503.

Weuster-Botz, D., V. Pramatarova, G. Spassov, C. Wandrey (1995). Use of a genetic algorithm in the development of a synthetic growth medium for *Arthrobacter simplex* with high hydrocortisone Δ›-dehydrogenase activity. *J. Chem. Technol. Biot.* **64**: 386-392.

Wylock, Chr. E., Colinet, Th. Cartage, B. Haut (2008). Coupling between mass transfer and chemical reactions during the absorption of CO_2 in a $NaHCO_3$-Na_2CO_3 brine: Experimental and theoretical study. *Int. J. Chem. React. Eng.* **6**: 1-20.

Yanagi, M., Y. Watanabe, H. Saiki (1995). CO_2 fixation by *Chlorellu sp. HA-l* and its utilization. Energy Convers. *Manage*. **36**: 713-716.

Zuzek, M., J. Friedrich, B. Cestnik, A. Karalic and A. Cimerman (1996). Optimization of fermentation medium by a modified method of genetic algorithms. *Biotechnol. Tech*. **10**: 991-996.