

## Agent based simulation of mixed algal cultures (two and three species) using biogas as CO<sub>2</sub> source

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### Abstract

Algae have recently received a lot of attention as a new biomass source for the production of renewable bio fuels. CO<sub>2</sub>, a green house gas is necessary for the culturing of microalgae. Algal systems can remove CO<sub>2</sub> from biogas. The main aim of this work is to study the interaction among the various factors affecting the growth rate and lipid production of different micro alga species from CO<sub>2</sub> for the production of bio fuels. In this work the microalgae were modelled as independent agents. CO<sub>2</sub> is assumed as the source of carbon for the growth and reproduction of microalgae. The effect of CO<sub>2</sub> concentration, energy utilization and saturation constant (for CO<sub>2</sub> utilization) was modelled in NetLogo software. Lipid production rate was also modelled. A comparison of how three different species behaved with respect to these variables was done by running the model with assigned selected values for these variables. This modeling can help in choosing the microalgal species combination for stable production. The effect of two species and three species were studied. The effect of saturation constant for CO<sub>2</sub> utilization had large effect in the yield of species in both two and three species combination model.

**Keyword:** NetLogo, Agents, Modeling, Microalgae, Lipids, Biofuels.

### INTRODUCTION

Algae have recently received a lot of attention as a new biomass source for the production of renewable energy. Micro algae with high oil productivities are desired for producing bio diesel [1]. The total oil and fat content of micro algae ranges from 1% to 70% of the dry weight [2]. The percentage of total lipid as neutral lipid, glycolipid, and phospholipid also varies widely among and within groups of microalgae [3]. According to the previous work, it was found that different algae species have different CO<sub>2</sub> tolerance level [4]. Some microalgal species which are having the CO<sub>2</sub> tolerance of 15% to 45% is shown in Table I [4]. Reduction of the emissions of CO<sub>2</sub> to the atmosphere can be addressed through biological CO<sub>2</sub>

mitigation which can further lead to the extensive uses of biofuel [2]. Some of the main characteristics which set algae apart from other biomass sources are that algae can have very high biomass yield per unit of light and area, can have a high oil or starch content, yet do not require agricultural land.

**Table I:** CO<sub>2</sub> Tolerance of Different Species [4]

Algae species	CO <sub>2</sub> tolerance
Euglena gracilis	45 %
Chlorella sp	40 %
Eudorine sp.	40 %
Dunaliella tertiolecta	20 %
Nannochloris sp.	15 %

They also not necessarily require fresh water as nutrients can be supplied by wastewater and CO<sub>2</sub> from combustion or biogas. Production of renewable energy with low emission of carbon can lead to the reduction of green house effect [5].

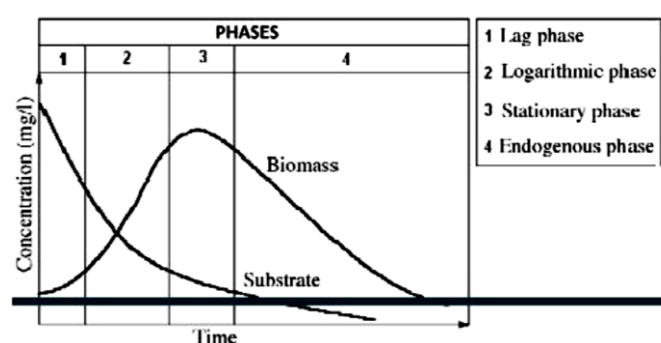
The use of micro algae for biological CO<sub>2</sub> sequestration has been considered. These include algae that contain the pigment chlorophyll and use the Calvin Cycle to fix carbon autotrophically. A number of marine algae species have been tested for CO<sub>2</sub> sequestration applications [6].

Chlorella minutissima was batch cultured at the high glycerine concentration and showed lipid production [7]. A modeling based on algal Productivity was done to describe the production of green microalgae (Scenedesmus obliquus and Coelastrum sphaericum) in a mass cultures [8]. This model was calibrated against 16 months of temperature and irradiance measurements with surface areas of up to 263 m<sup>2</sup>. Chlorella minutissima UTEX2341 growth and lipid production under photoheterotrophic fermentation conditions was done [7].

The growth processes of *Chlorella minutissima* were same fitted to Monod & Logistic equations. The equation is:

$$\frac{dx}{dt} = \mu \max \left( 1 + \frac{x}{x_{\max}} \right)$$

Where  $dx/dt$  is the rate of microalgae growth;  $\mu_{\max}$  is the maximum specific growth rate of the microalgae;  $X$  is the concentration of microalgae in the medium;  $x_{\max}$  is the maximum value of the microalgal concentration. Agent based modeling of an activated sludge process in a batch reactor Netlogo software was done by [9]. This work was done to study the feasibility of microalgae growth using agent based modeling to study the activated sludge process [9]. The concentrations of substrate and algal biomass in an activated sludge batch reactor is shown in Figure 1



**Figure 1.** Concentrations of substrate and algal biomass in batch process [9]

Lipids are one of the main components of micro algae; depending on the species and growth conditions 2 to 60 percent of total cell dry matter as membrane components, storage products, metabolites and storages of energy [3].

Mostly microalgae were cultured for the production of biofuels by using atmospheric or external  $\text{CO}_2$  source. But in this work, microalgae growth was simulated by using  $\text{CO}_2$  from biogas as a source. Due to this,  $\text{CO}_2$  is eliminated from biogas and gives us purified methane from biogas. This also leads to the growth of microalgae for the production of biofuels. The main aim is to study the interaction among the various factors affecting the growth rate and lipid production of different micro algae species using agent based modeling.

### Motivation for modeling

Current cost for production of micro-algal biomass range of \$8 - 15 /kg for ash-free organic dry biomass [10]. It was asserted that the capital and operating cost of the paddle wheel-mixed high-rate pond system for microalgae biomass production would be above \$ 100,000/ha for biofuels production alone. For algal biomass to be cost competitive with petroleum-derived fuel stocks, the cost of production needs to be reduced [10]. So, there is also a need to optimize the trade-off between biomass growth and lipid production.

### Agent Based Modeling

In agent-based modeling (ABM), a system is modelled as a collection of autonomous decision-making entities called agents. It is one of the most exciting practical developments in modeling for simulating the actions and interactions of autonomous agents with a view to assessing their effects on

the system as a whole. NetLogo is a freely available programmable modeling environment for simulating natural and social phenomena. It is used to create and open simulations and play with agents, exploring their behavior under various conditions. In this software, agents are beings that can follow instructions. Sliders are used in this software for changing the values of variables. Mobile agents or microalgae is treated as turtles which is moved over a grid of stationary agents or the unit of area which is called as patches.

The system modeled in this work is a batch bioreactor of 1 litre volume. The vessel is initially filled with biogas at atmosphere (correspond to 0.6875 gm of  $\text{CO}_2$ ). Algae consume the carbon dioxide content present in the biogas for its growth thus providing us with purer form of biogas which can be easily utilized for various purposes.

The relationship of specific growth rate to substrate concentration often assumes the form of saturation kinetics. The kinetics is usually described by the Monod equation.

### Model of $\text{CO}_2$ Utilization

The dissolved  $\text{CO}_2$  concentration in the biomass system was 0.6857 gm. As per Calvin cycle in the photosynthesis process, 6 molecules of carbon dioxide lead to the formation of 1 molecule of glucose through the fixation of carbon dioxide to 12 molecules of triose phosphate. This molecule of glucose then undergoes glycolysis that converts glucose to pyruvate. In glycolysis overall 7 ATP are produced per glucose, which traces back to 6 molecules of carbon dioxide. We know that one mole of ATP releases 45.6 kJ of energy. So for ATP per 6 molecule of  $\text{CO}_2$  a species can get 1.21 kJ of energy. Different species of algae have different capacities for carbon fixation. Carbon dioxide concentration of each patch decreases with every tick as the algae utilize dissolved carbon dioxide from the medium for their movement and growth.

To achieve the first target of this project work various parameters were consider and some data were assumed to tune the simulation:

- As in biogas, the percentage of  $\text{CO}_2$  is 35%. So,  $\text{CO}_2$  concentration is assumed 35% and was set as 0.6875 g/l.
- It is assumed that  $\text{CO}_2$  is the only energy source for the system and no additional energy is supplied.

In this work, the effect of three parameters i.e.  $\text{CO}_2$  utilization and energy for doubling and saturation constant for  $\text{CO}_2$  utilization explain the algae growth rate. For two species and three species combination, three variables were studied with two levels of these variables. High (H) is 75% of the maximum value, where as low (L) is 25% of the maximum

value. Three cases for each species shows the effects of these variables on the growth or yield of algal species. The simulation was run by Plackett-Burman design, Table III and II shows the summary of the different cases with three variables and two levels for three species and two species combination. Each horizontal row represents a trial and each vertical column represents the H (high) and L (low) values of one variable in each trials. This design requires that the frequency of each level of a variable in a given column is equal and that in each test (horizontal row) the number of high and low variables should be equal.

## RESULTS

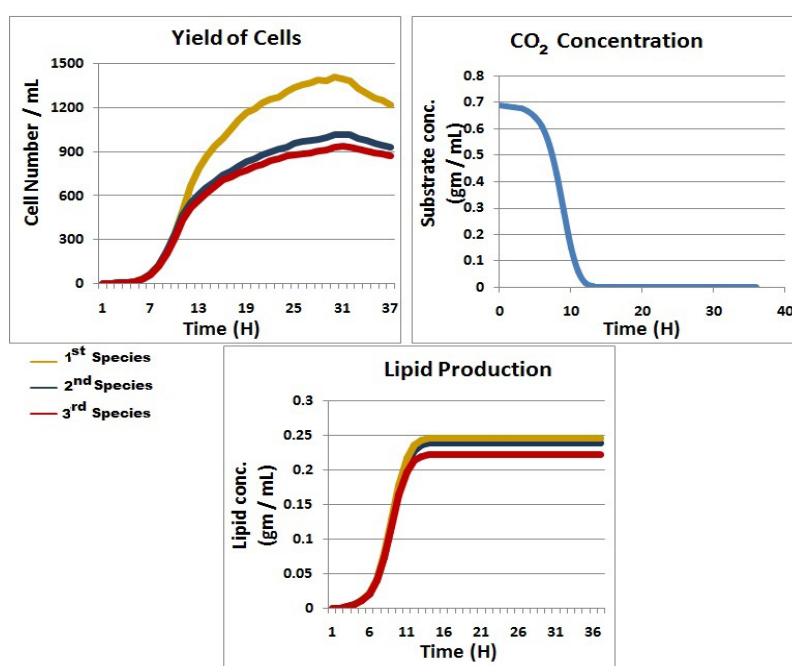
To simulate algal growth simulation, a set of experiments were done. Eighteen cases with average as per Plackett Burman factorial design shows the effect of three variables on each species (CO<sub>2</sub> utilization, energy require to double and saturation constant for CO<sub>2</sub> utilization for each species) on the growth of micro algal species. Another twelve cases were summarized in Table II which shows the effect of these variables for two species combination. Previously in conference proceeding two species was modeled with factorial design model. The yield of cells at different variable value for two species is summarized in Table II.

**Table II.** Factorial Design Model of three Variables and two Level for two Species

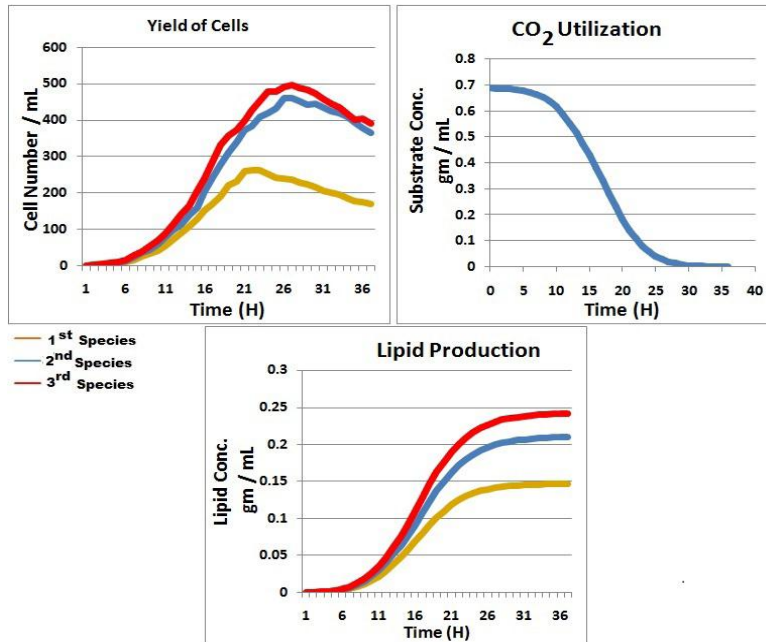
Sr. No	Energy Utilization		CO <sub>2</sub> Utilization		K value		Yield of cells	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
1	L	H	H	H	H	H	1721	1183
2	H	L	H	H	H	H	1159	1663
3	H	H	L	H	H	H	1008	1795
4	H	H	H	L	H	H	1643	784
5	H	H	H	H	L	H	231	1686
6	H	H	H	H	H	L	1796	127
7	H	L	L	L	L	L	310	470
8	L	H	L	L	L	L	766	139
9	L	L	H	L	L	L	214	229
10	L	L	L	H	L	L	296	554
11	L	L	L	L	H	L	2652	109
12	L	L	L	L	L	H	108	2525

### A. Effect of Energy required for growth

The algae require energy to live and grow. Some species may need more than other. The effect of this differential requirement of energy is studied in cases 1-3 and 10-12. Clearly as the energy utilization goes from H to L the growth rate goes of the corresponding species is much higher compared with other species. The number of species and growth curve with different energy utilization level of each species is shown in Figure 2 and 3.



**Figure 2.** Growth curve and lipid production at different energy utilization value of one species and rest species with high level of variables based on case 1



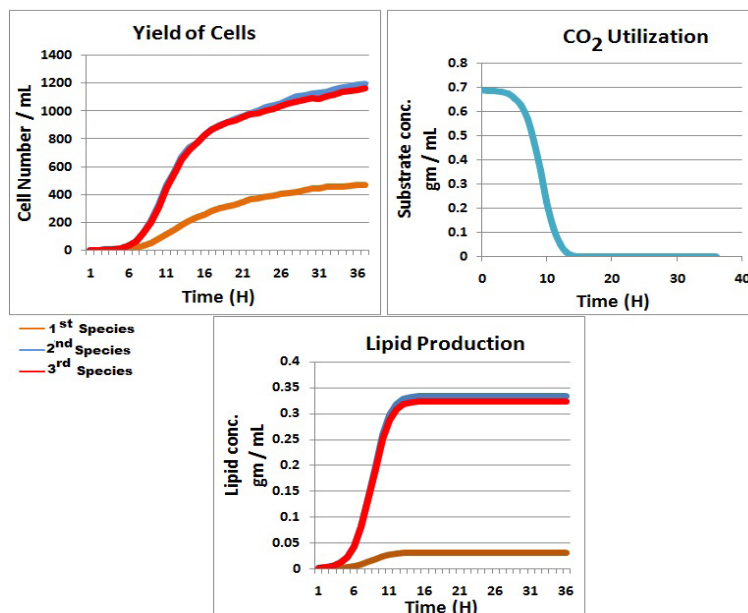
**Figure 3.** Growth curve and lipid production at different energy utilization value of one species and rest species with low level of variables based on case 10

#### B. Effect of CO<sub>2</sub> Utilization

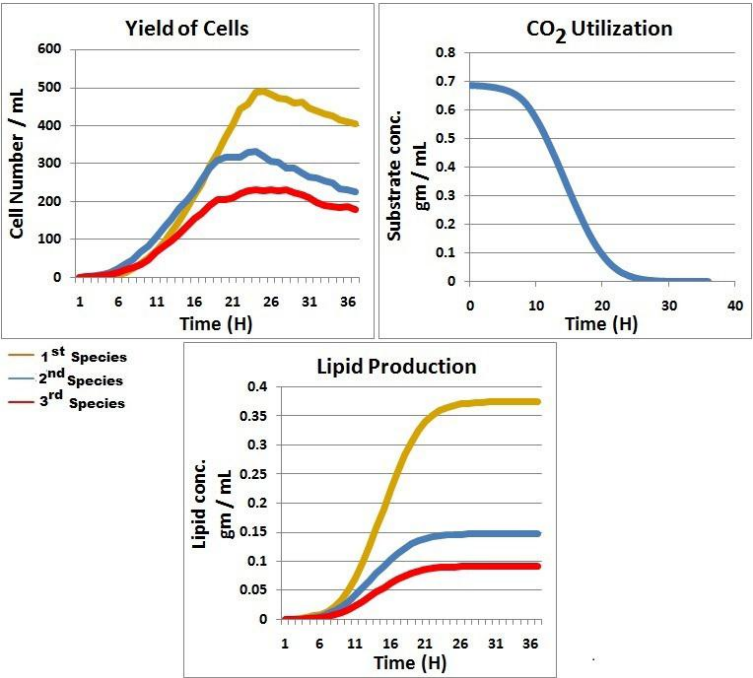
The algae require CO<sub>2</sub> as a source of nutrient to live and grow. Different species are having different CO<sub>2</sub> utilization range. The effect of this differential utilization of CO<sub>2</sub> is studied in cases 4-6 and 13-15. Clearly as the CO<sub>2</sub> utilization goes from H to L the growth rate goes of the corresponding species decreases as compared with other species. The number of species and growth curve with different CO<sub>2</sub> utilization level of each species is shown in Figure 4 and 5. In Figure 4 faster rate of CO<sub>2</sub> utilization comes as compare to 5 due to the high level of CO<sub>2</sub> utilization and saturation constant value.

#### C. Effect of saturation constant for CO<sub>2</sub> utilization in the number of species

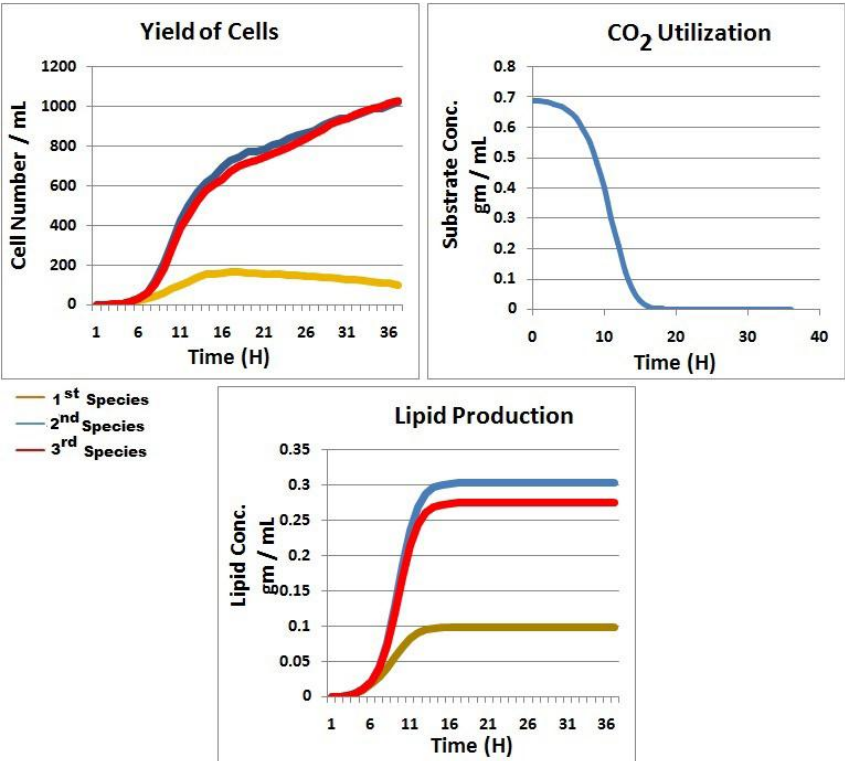
The effect of saturation constant value in the number of species and growth curve each species is shown in Figure 6 and 7. As per the model simulated, species with low level of k value gives less number of yields as compared to the species with high level of K value.



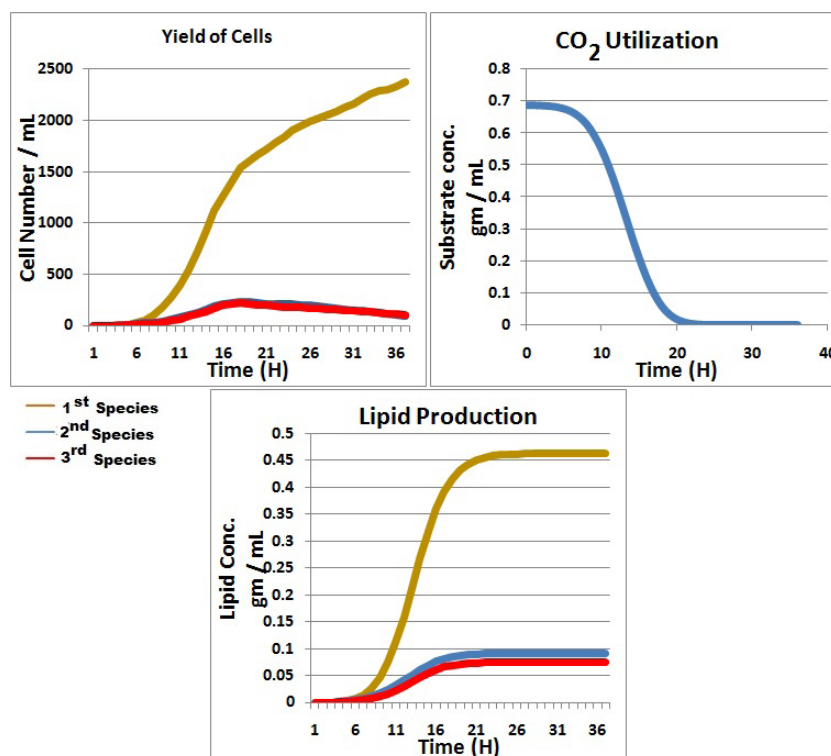
**Figure 4.** Growth curve and lipid production at different CO<sub>2</sub> utilization value of one species and rest species with high level of variables based on case 4



**Figure 5.** Growth curve and lipid production at different CO<sub>2</sub> utilization value of one species and rest species with low level of variables based on case 13



**Figure 6.** Growth curve and lipid production at different saturation constant value of one species and rest species with high level of variables based on case 7



**Figure 7.** Growth curve and lipid production at different saturation constant value of one species and rest species with low level of variables based on case 16

## DISCUSSION

CO<sub>2</sub> is utilizing faster as shown in Figure 2, 4 and 6 because of high level of CO<sub>2</sub> utilization and saturation constant value and results in more number of species. Whereas, in Figure 3, 5 and 7 less number of species with delayed CO<sub>2</sub> utilization is due to the low level of CO<sub>2</sub> utilization and saturation constant value. Exponential death started of species 1st in Figure 3 due to the high level of energy required to reproduce and low level of CO<sub>2</sub> utilization and saturation constant value. Saturation

constant for CO<sub>2</sub> utilization is having high impact in the yield of cells. Its high level gives large number of cells as compare to its low level which is shown in Figure 6 and 7.

Yield of cells using CO<sub>2</sub> as a source of nutrient in these cases is summarized in Table III. The stages in analyzing the data of Table II and III were done using Nelson's method. The effect of an independent variable on the response is the difference between the average response at the high level and the average value at the low level.

**Table III.** Factorial Design Model of three variable and two level for three species combination

Sr. No	Energy Utilization			CO <sub>2</sub> Utilization			K value			Yield of cells		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
1	L	H	H	H	H	H	H	H	H	1226	931	876
2	H	L	H	H	H	H	H	H	H	988	1296	902
3	H	H	L	H	H	H	H	H	H	945	802	1338
4	H	H	H	L	H	H	H	H	H	467	1196	1168
5	H	H	H	H	L	H	H	H	H	1310	564	1384
6	H	H	H	H	H	L	H	H	H	1267	1324	456
7	H	H	H	H	H	H	L	H	H	98	1023	1028
8	H	H	H	H	H	H	H	L	H	1307	72	1316
9	H	H	H	H	H	H	H	H	L	1154	1161	56
10	H	L	L	L	L	L	L	L	L	169	364	389
11	L	H	L	L	L	L	L	L	L	391	151	415



12	L	L	H	L	L	L	L	L	L	451	442	118
13	L	L	L	H	L	L	L	L	L	403	223	177
14	L	L	L	L	H	L	L	L	L	223	376	215
15	L	L	L	L	L	H	L	L	L	252	211	374
16	L	L	L	L	L	L	H	L	L	2377	98	109
17	L	L	L	L	L	L	L	H	L	105	2496	115
18	L	L	L	c	L	L	L	L	H	136	93	2279

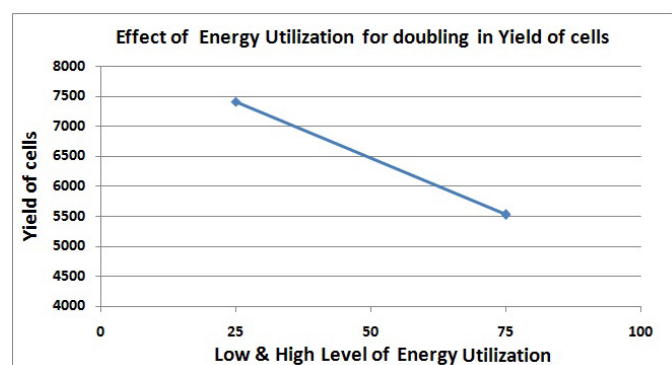
**Table III.** Factorial Design Model Of Three Variable And Two Level For Three Species

Factor									
Effect of Variables for three species combination									
	Energy Utilization			CO2 Utilization			K Value		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
ΣH	7705	7224	7304	8698	1881	8442	11041	10793	10747
ΣL	5564	5599	5411	4571	4642	4273	2228	2030	1968
Difference	2141	1625	1893	4127	3539	4169	8813	8763	8779
Effect	237	180	315	458	393	463	979	973	975
Effect of Variables for three species combination									
	1 <sup>st</sup>	2 <sup>nd</sup>		1 <sup>st</sup>	2 <sup>nd</sup>		1 <sup>st</sup>	2 <sup>nd</sup>	
ΣH	1647	5714		6967	7008		9979	9636	
ΣL	5933	5608		5240	4526		2128	1898	
Difference	214	106		1827	2482		7851	7738	
Effect	35	17		121	86		206	159	

Thus the effect of each variable is calculated as

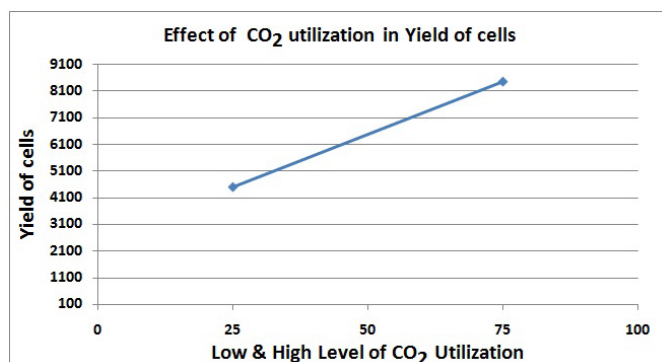
$$\frac{\sum H}{nH} - \frac{\sum L}{nL}$$

Simulated data showed a linear relationship between lipid accumulation and algal growth. Effect of saturation constant for CO<sub>2</sub> utilization has a large effect and its effect in the yield of species also goes on increasing from two species combination to three species combination.

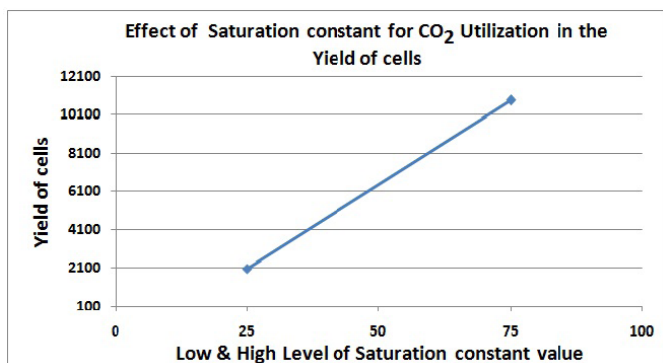


**Figure 8.** Effects of high and low level of Energy Utilization in the yield of species

Based on the analysis, effect of the variable on yield of the species is shown in above figures. The observed substrate consumption dependent biomass production suggests that CO<sub>2</sub> concentration most likely regulates the growth pattern of microalgae and was done by the model developed under this study.



**Figure 9.** Effects of high and low level of CO<sub>2</sub> utilization in the yield of species



**Figure 10.** Effects of high and low level of saturation constant value of CO<sub>2</sub> utilization in the yield of species

## CONCLUSION

The model describes all the actions of the algae as agents and their respective interactions with their surroundings and dynamics emerge from this. Microalgae metabolism is a complex process and varies in terms of substrate concentration which is CO<sub>2</sub>, energy utilization for doubling and saturation constant. Agent based modeling allows the researcher to develop a bottom up approximation to study the CO<sub>2</sub> dependent mixed growth microalgae culturing in a batch process. The effect of each variable on the microalgae culturing is shown in Table IV. It is found that saturation constant for CO<sub>2</sub> utilization had large effect which is very significant. This gives the optimized effects for the CO<sub>2</sub> utilization and saturation constant value. Furthermore, it was found that lipid production is microalgal growth associated. The model was applied to determine the optimum values of the parameters controlling and the choosing of species combination in the batch process. This information will be useful in the process scaling up and commercial biofuels production.

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