A COMPARATIVE STUDY OF BIOMASS AND LIPID ACCUMULATION IN DUNALIELLA SALINA AND CHLORELLA VULGARIS UNDER DIFFERENT NITROGEN SOURCES FOR BIODIESEL PRODUCTION

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Abstract

To evaluate the growth characteristics and lipid accumulation in unicellular green algal species Dunaliella salina and Chlorella vulgaris as a feedstock for biodiesel production by using different nitrogen sources. This study designed for environmental friendly and cost effective approach with kinetic modeling of optimization for algal growth using different nitrogen sources (Urea, Peptone and Yeast extract). The concentration of nitrogen sources was in the range of 0.5gm to 2.5gm and the culturing medium was Walne's medium(D.s) & Bold Basal Medium (C.v) with an initial pH of 7 at a temperature of 28^{o}C with illumination of 1000 Lux and aeration provided by an aerator pump 10hrs light/14hrs dark ration in a laboratory. The biomass concentrations of 2.44gm/250ml, 1.598gm/250ml, 2.4gm/250ml was obtained for 1.5gm of urea, 2.0 gm of peptone and 2.5gm of Yeast extract respectively. Lipid productivity in the ranges was 2.16 g/l of urea, 1.05 g/l of peptone and 0.79 g/l of Yeast extract respectively. Hence, maximum biomass concentrations of 2.44g/l, urea were used as the source of nitrogen. Thus, the kinetics of logistic model and leudeking-pirot model is an efficient technique of optimization of biomass concentration and lipid product. The efficient technique for finding biomass was kinetic modeling of logistic model, growth and non-growth associated parameter measured by Ledeuking pirot model. The result shows that urea performed high yield of biomass concentration with lipid production. The experimental values were fitted with modeling parameters and it can be used as potential sources of nitrogen for increasing lipid production.

Keywords: Kinetics – logistic modeling and Leudeking pirot model, Lipid induction, Biomass concentration, Nitrogen sources-Urea, Peptone and Yeast extract.

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Introduction:

Increasing population consumes the large energy from fossil fuel and it creates demands of fuel and environmental pollution in the world. Research ideas are developed now-a-days regarding fuel from naturally available sources (Biomass from waste, plant sources) that production costs are minimal and environment friendly for producer and user (Amitkumar Sharma et al., 2015), Among the other source of biomass, microalgae are aquatic photosynthesis plants that reduces the cost of production. Lipid content is accumulated by using nitrogen as the limiting source under stress condition (Rajasri yadavalli et al.,2012). Cultivation of microalgae under different environmental and nutrient conditions such as, light intensity from autotrophic and heterotrophic, Co_2 absorption, nutrient from various sources, medium in which algae grows can improve the lipid accumulation in the microalgae (Y. Li., et al, 2015). Nitrogen (controlled nutrients) plays a major role in increasing or decreasing production of algal growth. Compositions of C/N ratio in phytoplankton are 5-20. Excessive/depleting nitrogen sources present in the medium can affect the starving of algae, which then reduces the rate of cell division.(Madura Sarkar). Nitrogen can adopt the environmental fluctuation like light, salinity & nutrients it can change the chemical composition, Degradation & turnover of nutrients can be converted into the recycling of nitrogen sources for phytoplankton metabolism.(J.A.Berges 2003). microalgae can get the energy from photosynthesis & nutrients from aquatic environments such as, aquatic plants, debris of marine species. The total nitrogen content of urea is 32% it a combined form of nitrogen source and Nitrogen released from decomposed seaweeds in the ranges are 5-6% and seaweeds contains about 60% trace elements (DennisHannisak 1993) .Even though the light energy & aeration is provided into the culture medium nutrient play an important role in the growth mechanism of algae. To overcome deficiency of all other nutrients from aquatic environment, nitrogen is the primary source for recycling of nutrients in aquatic plant life. The high amount of oil content produced more than 30% v/v from microalgae as a renewable source than the other source of feedstock such as, from crop varieties stated by (Demirbas.A 2010). Extraction of algae biomass from a liquid culture medium by ultrasonication generates Sound wave with alteration of pressure cycle this release the lipid content present in the algae (Gulab Chand shah et al). Growth and lipid content o*f chlorella* strains *C.emersonii, C.minutissima &C.Vulgaris* increased the lipid content with decreasing concentration of nitrogen & Temperature in the ranges were 63%,56% & 40% of DCW stated by (illman et al). The maximum specific growth(μ_{max}) And Monod or Saturated constant(K) can be calculated by simple method of kinetic modeling is Monod growth kinetics [18].Characteristics of cell growth in a culturing period and its parameters can be evaluated by logistic growth kinetics and product formation regarding growth and non growth associated parameters can be evaluated by ledeuking pirot kinetics.

The present research work focused on development of simple kinetic models (Monod, Logistics and Leudeking –pirot) for lipid production using algal biomass with optimized parameters in batch culture process.

Materials and Methods:

Algal strain: Unicellular strains of *Dunaliella salina, Chlorella vulgaris* purchased from CMFRI Central Marine Fisheries Research Institute, Tuticorin. This culture maintained in 4^{o}C in a freezer. Before inoculation of culture, stock cultures were placed to set to attain a normal temperature.

Medium preparation: Nutrients used for *Dunaliella Salina* were Walne's medium, which is prepared in a laboratory itself.

Walne's medium Composition: Solution A (1 ml/liter of seawater)-:

Solution A: Fecl.6$H_2$0 -1.3gm, Mncl$_2$.4H20-0.4gm H_3B0_3 -33.6gm, Na2EDTA-45gm, NaH2P04.2H20-20gm. Solution B -1 ml. **Solution B per100ml:-** Zncl2-2.1gm, $(NH_4)_6$Mo7$H_2$0.4$H_2$0-0.9gm, $(NH_4)_6$Mo7$H_2$0.4$H_2$0 - 0.9gm, cuso4.5$H_2$0 - 2gm, Concentrated Hcl-10ml. **Solution C per 200ml(0.1ml/100ml):** Thiamine Hcl(vit B_1)-1ml, cyanocobalamin (vit B_{12})-10mg.**Solution D per litre:** for diatoms only(2ml)- Na_2sio_3.5$H_2$0 - 40gm.

Bold Basal Medium Composition: Nutrients used for *Chlorella vulgaris* were Bold Basal Medium which is prepared in an laboratory itself. Preparation of Bold Basal Medium by (Daphnia Research Group)

Different sources of nitrogen:

Sources of nitrogen used in this work were Urea, Peptone and Yeast extract in the ranges from 0.5gm,1gm,1.5gm,2.0gm & 2.5gm respectively.

Subculturing of *Dunaliella salina, Chlorella vulgaris*:

Stock culture of *Dunaliella salina, Chlorella vulgaris* were grown in the petriplates for one week under the LED light using algae culture agar (Himedia lab) with Walne's and BBM medium respectively. Exponential phase of growing algae was used for culturing and analysis part.

Experimental condition of culturing algae:

Vessels and medium used for this work is autoclaved at 121^{o}C for 15min. 250ml of the fifteen Erlenmeyer flask taken and each nitrogen were source added into the each five flask respectively. The indoor system carried out using 10ml inoculums of *D.S* & *C.V* in 100ml ml of culture medium with varying source of nitrogen with different concentrations, keeping other nutrients and parameter as constant PH 7 and Temperature of 28oC was maintained throughout, and illumination of fluorescent light, aeration was provided continuously throughout the incubation period of 24 days. The effect of various

concentrations of nitrogen sources on cell concentrations corresponding yield of lipid was investigated. Seawater collected which was filtered, sterilized, and added, into the growth medium for algae growth.

Measurement of Biomass concentration:

Biomass concentration was measured by taking Optical Density of culture at 640nm by Bio spectrophotometer for every 24 hours after that same culture proceed for cell counting by Heamo cytometer coated with a tryphan Blue dye used for identifying the live cells and non living cells in the microscope. Similarly, cells are centrifuged (15000 Rpm) in a high speed research centrifuge the resultant pellet are washed with double distilled water and weighed for initial biomass concentration and dried in an oven at 105^{o}C weighed for final biomass concentration at the period of first day, similarly the biomass concentration was calculated every day up to the incubation period. Also the cells can be dried by a sun shade drying.

The growth rate was measured by

$$\text{Growth rate } (\mu) = (\ln x_t - \ln x_o)/T_t - T_o \quad (1)$$

Where X_t, X_o were dry cell weight at that time of final and initial time respectively.

Extraction of algal oil:

Shade dried algal biomass retain their natural structure with lipid content. 25gram of dried algal biomass was mixed 50ml of ether solution in a beaker container with continuous ultrasonic treatment for 20min.Fluctuating pressure in an algae solution create the shear stress to cells and breaking of cell wall which releases the lipid content. Oil has to be further recovered by screw press, which is further used for transesterification process. (Oilgae, Gulab Chand Shah et al).

The lower organic layer with the lipids transferred to a clean pre-weighed Vail (W1). Evaporation was carried out in hot air oven at 80^{o}C of the sample was again weighed (W2).

$$\text{The lipid content was calculated by L (\%)} = W_L/W_B \quad (2)$$

Where, W_L – weight of the extracted lipid before drying, W_B –weight of the extracted lipid after drying.

The Biomass productivity P_B calculated by the equation

$$P_B = P_B = (W_{BF} - W_{BO})/t \quad (3)$$

The lipid productivity P_L was calculated by, $P_L = P_B * L(\%)$ (4)

Logistic Modeling for growth kinetics:

In a batch culture reaction, growth rate is directly proportional to cell concentration, which is given by a mathematical model,

$$\frac{dx}{dt} = \mu x$$

And logistic equation Baileys and Olis (1968)

$$\frac{dx}{dt} = kx(1 - x/xs)$$

On integrating give,

$$x = \frac{X_m}{1+(X_m))-1)e^{-\mu t}} \quad (5)$$

Where, x - concentration of algal biomass concentration, t – biomass concentration at a time, μ - specific growth rate. x_s - biomass concentration in stationary phase.

Kinetic model for Lipid production:

The most accurate method of outputs like energy, growth and Non-growth associated product can be subscribed by the kinetics named as Leudeking - piret model (Kinetic for product formation Leudeking,piret model 1959), this outcome of product formation linear to that of biomass concentration and growth rate. This is written by the equation,

$$\frac{dp}{dt} = \alpha\frac{dx}{dt} + \beta x \quad (6)$$

α ,β are the kinetic constant of product formation. The value for β obtained by plotting the graph of dx/dt at stationary phase.

$$\beta = \frac{dP/dt}{X_{max}} \quad (7)$$

X_{max} which gives,

$$P_t = P_0 + \alpha X_0 \left\{ \frac{e^{\mu t}}{\left(1-\frac{X_0}{X_m}\right)(1-e^{\mu t})} - 1 \right\} + \beta \frac{X_{max}}{\mu ln\left(1-\frac{X_0}{X_m}\right)(1-(e^{\mu t}))}$$

$$P_t - P_0 - \beta \frac{X_{max}}{\mu ln\left(1-\frac{X_0}{X_m}\right)(1-e^{\mu t})} = \alpha X_0 \left[\frac{e^{\mu t}}{\left(1-\frac{X_0}{X_m}\right)(1-e^{\mu t})} - 1 \right] \quad (8)$$

By plotting of Y vs X gives the slope of α value,

Amount of product produced by growth associated Pα(U/ml) & non-growth associated , P_β

$$P_\alpha = \alpha.Xo \frac{e^{\mu t}}{1-\left(\frac{X}{Xmax}\right)1-e^{\mu t}} \quad (8)$$

$$P_\beta = \beta \left(\frac{Xmax}{\mu}\right) \ln\{1-\left(\frac{Xo}{Xmax}\right)(1-e^{\mu t}) \quad (9)$$

Table: 1 Biomass concentration and Lipid production in *Dunaliella salina* using various nitrogen substrates

S.no	urea (gm)	urea (x) (gm/250ml)	Lipid productivity Urea (P) (gm/250ml)	Peptone conc S_P (gm)	Biomass conc Xp (gm/250ml)	Lipid productivity Pp (gm/250ml)	Yeast extract S (gm)	Biomass conc yeast X (gm/250ml)	Lipid productivity Py (gm/250ml)
1.	0.5	2.05	2.23	0.5	0.75	2.67	0.5	0.14	0.056
2.	1.0	2.23	6.90	1.0	0.85	2.73	1.0	0.19	0.17
3.	1.5	2.44	6.01	1.5	1.09	3.38	1.5	0.24	0.232
4.	2.0	1.99	5.54	2.0	0.68	1.29	2.0	0.31	0.62
5.	2.5	1.85	3.14	2.5	0.36	1.46	2.5	0.34	0.527

Table: 2 Biomass concentration and Lipid production in *Chlorella vulgaris* using various nitrogen substrates

S.no	Urea conc (S) (gm)	Biomass conc urea (x) (gm/250ml)	Lipid productivity Urea (P) (gm/250ml)	Peptone Conc (S) (gm)	Biomass conc (x) (gm/250ml)	Lipid productivity Urea (P) (gm/250ml)	Yeast extract (S) (gm)	Biomass conc urea (x) (gm/250ml)	Lipid productivity Yeast extract (P) (gm/250ml)
1.	0.5	1.41	2.68	0.5	0.86	2.13	0.5	0.29	0.13
2.	1.0	1.13	2.62	1.0	1.32	2.82	1.0	0.63	0.75

3.	1.5	1.385	2.15	1.5	1.52	2.15	1.5	0.82	0.75
4.	2.0	1.31	2.46	2.0	1.598	2.42	2.0	0.84	1.661
5.	2.5	1.21	1.17	2.5	0.75	1.17	2.5	1.27	3.02

Table: 3 Kinetic parameters of Monod groth and Logistic growth and Leudeking pirot modeling for *Dunaliella salina* and *chlorella vulgaris*:

Species	Logistic modeling K	R	Leudeking modeling α	β	Monod growth modeling μ_{max}	K_s	Q_x	Q_p	Y($\frac{p}{X}$)	Y($\frac{p}{s}$)
D.S urea	1.46	0.914	0.2172	0.5	0.068	1.46	1.734	1.233	0.175	0.193
D.S peptone	0.551	0.912	0.1211	0.178	0.041	1.5	1.521	1.083	0.154	0.142
D.S pl.trmgs	0.31	0.899	0.179	0.118	0.031	2.5	1.017	0.832	0.023	0.015
CV urea	0.83	0.905	0.104	0.328	0.066	2.1	1.113	0.312	0.027	0.123
CV pep	0.52	0.891	0.15	0.04	0.05	1.36	1.239	0.938	0.147	0.129
CV pl.trmgs	0.362	0..784	0238	0.109	0.082	1.3	1.002	0.108	0.012	0.008

Results and Discussion:

Algal strains of *Dunaliella salina and Chlorella vulgaris* were cultivated in the culture medium of Walne's and BBM with various sources of nitrogen such as urea, peptone and yeast extract in the concentration ranges 0.5gm,1gm,1.5gm,2gm,2.5gm respectively. Culturing of algal strains with nitrogen leads to increase the biomass concentration from the 4th day of Incubation period. Fig 1 Results *D.s* strain attains the maximum biomass concentration of 2.44gm/250ml in the 18th day of incubation using 1.5gm of urea as the nitrogen source. Similarly the maximum biomass concentration of *C.v* was 1.598gm/250ml using 2gm peptone as the nitrogen source in the 12th day of Incubation period in the Fig 2. Fig 3 represents increasing concentration of nitrogen sources in the exponential phase leads to increases the

lipid content of 23.24% for *D.s* and 21.67% for *C.V* . After increasing the nitrogen sources in the stationary phase leads to produces the lipid with accumulation of some dead cells which affect the % of lipid production fig.3 resulted by increasing concentration lipid concentration was reduced 10.12 gm/250ml for 2.5 gm of urea and 8.11 gm/250ml for 2.5 gm of peptone. This study also revealed about kinetic modeling of growth and product formation i.e., Monod growth kinetics, Logistic growth and Leudeking product formation kinetics parameters were found to fit the experimental values. Table 1 refers the lipid productivity was calculated based on Eqn.3 and the maximum lipid productivity for D.S 6.9 gm/250ml using 1gm of urea and highest lipid productivity in the C.v was 2.82 gm/250ml using 0.5 gram of peptone as a nitrogen source. Monod growth of yield coefficient of process and product $\{Y(\frac{p}{s})\}$ were 0.146, 0.193 for D.S and Yield coefficient for specific product yield $\{Y(\frac{p}{X})\}$ of D.S & C.V were 0.175 and 0.147 respectively. Maximum lipid production rate Q_P obtained 1.233 for D.S 1.5gm of urea & 0.938 for C.V 2gm of peptone with the corresponding specific growth rate (μ) 0.593 d^{-1} & 0.472 d^{-1} respectively. Volumetric cell mass production rate Q_X of D.S urea was 1.73 g/l & C.V peptone was 1.239 g/l. Monod /Saturated constant K_S of D.S urea was 1.46 and C.V Peptone 1.36. Hence, the results showed the biomass concentration directly related to that of lipid concentration. Simple growth and Non growth associated kinetic model is Logistic growth and Ledeuking pirot model. Growth associated value (α) for *D.s* is 0.2172 and for *C.v* is 0.15. Similarly, the non-growth associated value β of D.s urea was 0.5 and C.v peptone was 0.04.

Conclusion:

Monod model (1949) suggested that growth phase of culturing process with the nutrients have some kinetics constant it was selected to that of quantitative determination of cell growth. Logistic model used for examining cell growth in an incubation period. 1.5gm of *D.s* reached the maximum biomass concentration of 2.44gm in 250 ml corresponding lipid productivity 6.99 gm increased by 1 gm of urea.2gm of *C.v* increases the biomass concentration was 1.598gm and the lipid productivity increased by 0.5 gm of peptone.

Leudeking pirot model described the growth association constant (α) & Non-growth associated constant (β) values obtained were depends on the oil content of microalgae with biomass production. 2.16 g/l maximum lipid productivity obtained for *Dunaliella Salina* using urea as a nitrogen source. 2.82 g/l obtained in chlorella vulgaris using peptone as nitrogen source. Growth associated value α (lipid content) higher in D.s and non-growth associated value β more in C.v, Hence the kinetic model fitted for experimental values.

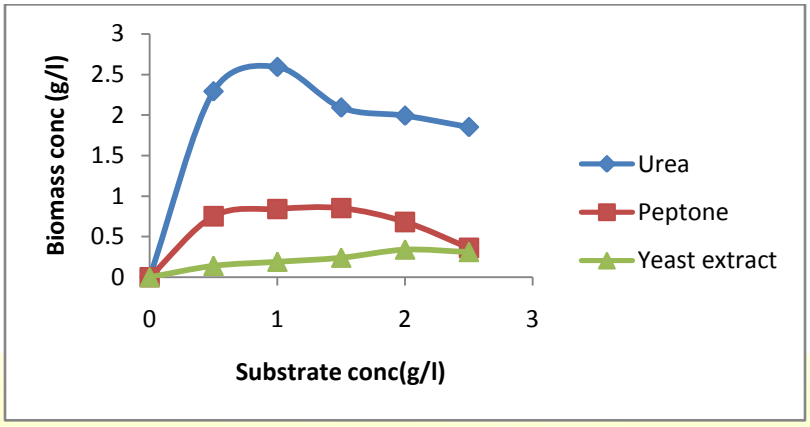

Fig 1: Biomass concentration of *Dunaliella salina* in a different nitrogen sources

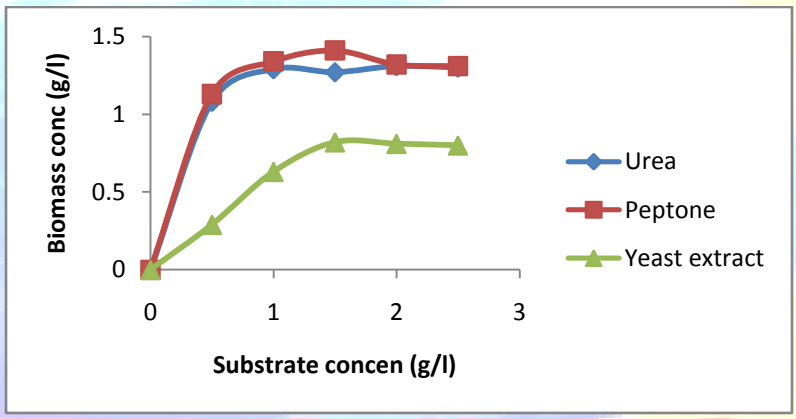

Fig 2: Biomass concentration of *Chorella vulgaris* in a various nitrogen sources.

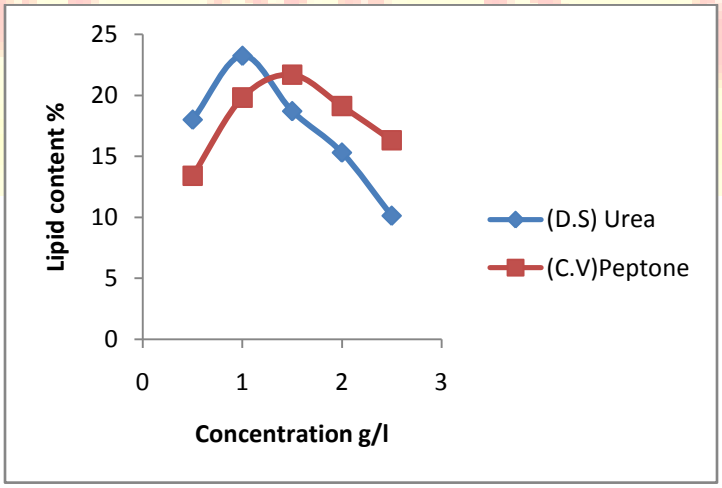

Fig 3: Lipid content % of D.S and C.V strains of different concentration ranges.

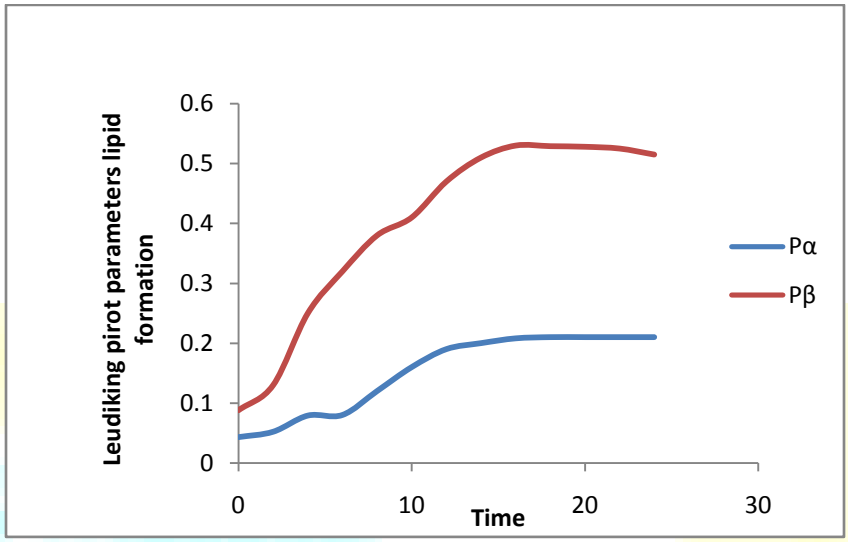

Fig 4: Growth and Non Growth parameter constant with corresponding time for D.S urea

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