Screening and Identification of Everglades Algal Isolates for Biodiesel Production

Priyanka Narendar [1], Miroslav Gantar[2], Krish Jayachandran[1]

Florida International University , Miami , Fl

[1]Dept of Earth and Environment

[2]Dept of Biological Sciences

Why Biofuels?

- Improve Energy security
- High Oil prices
- Mitigate climate change

Third Generation Biofuels

- Algal fuel or Oilgae
- 30-100 times more oil per acre than corn and soybeans
- No sulfur, non-toxic
- Grown in marginal land
- Biodegradable
- Less water consumption
- Carbon sequestration
- Tolerate brackish and saline waters

Crop	Oil Yield Gallons/acre
Corn	18
Cotton	35
Soybean	48
Mustard seed	61
Sunflower	102
Rapeseed/Canola	127
Jatropha	202
Oil palm	635
Algae (10 g/m²/day at 15% TAG)	1,200
Algae (50 g/m²/day at 50% TAG)	10,000

Source: http://www.dailymarkets.com/stocks/2008/07/02/investing-in-algae-biofuel/

Disadvantages:

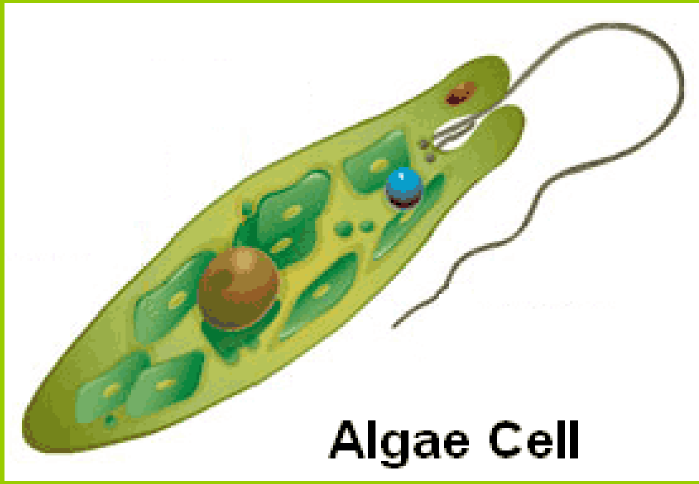
- Is it economically feasible?
- Major issues with harvesting and labor costs
- Contamination issues

Objectives:

- 1. To screen algal strains from the Florida Everglades to identify those with potential for biodiesel production.
- 2. Assess the effect of environmental conditions on accumulation of cell lipid.

Algae

- **Photosynthetic eukaryotes**
- **Lipid of interest: Neutral lipids (in the form of Triacyl glycerol) – best substrate for producing biodiesel.**



Organisms used:

- 31 algal strains from the FIU culture collection
- Reference strain: *Botyrococcus braunii* -

Genus	**Strain**
Chlamydomonas	EV 29
Chlorella	EV 2-4,71-4
Selenastrum	EV 2-7,34-4
Scenedesmus	EV 3-11, 66-1, 79-1, 80-15, 81-5, 103-4
Chlorococcum	EV 5-1, 45-3, 55-2, 55-5
Coelenstrum	EV 46-4, 108-5
Coccoid Green	EV 56-5, 56-4, 81-7, 103-6, 64-12
Stirgeoclonium	EV 64-8
Dactylococcus	EV 64-10
Pediastrum	EV 81-6, 104-6, 108-4
Prochlorococcus	EV 104-1a
Kirchneriella	EV 104-7

Culture conditions:

- **Algal biomass was produced by growing algal strains in 3-liter flasks in BG11 medium under cool white light (30µ E m^{-2} sec^{-1}) at 27°C with aeration with sterile air.**

Screening for lipids:

- **Nile Red Fluorescence technique (Greenspan et al.,1985).**

- **A lipophilic dye**
- **Spectroflurometer analysis (excitation 530 nm; emission 575 nm)**
- **Calibration Curve-Lipid standard**

  Triolein
- **Percentage dry weight determination**

Quantification of Lipid –Gravimetric technique

- Freeze dried algal biomass (1g)
- Solvent-Chloroform-methanol- water system
- Solvent evaporated using air
- Mass of lipid estimated gravimetrically

Assessment of the effect of environmental factors:

- **Biomass and Lipid accumulation**:
1. Determined over a 45 day period.
2. Biomass concentration
3. Lipid accumulation: Nile red method
- **Nitrogen Depletion**
1. Cells washed thoroughly with N free medium before transferred to fresh media.
2. Concentrations:0%, 50% and 100% of the standard nitrogen content in the BG 11 medium.
- **Phosphorous Depletion**
- Cells washed thoroughly with P free medium before transferred to fresh media.
1. Concentrations: 0%,50% and 100% of the standard phosphorous content in the BG 11 medium.

Results: Lipid concentration using Nile Red method

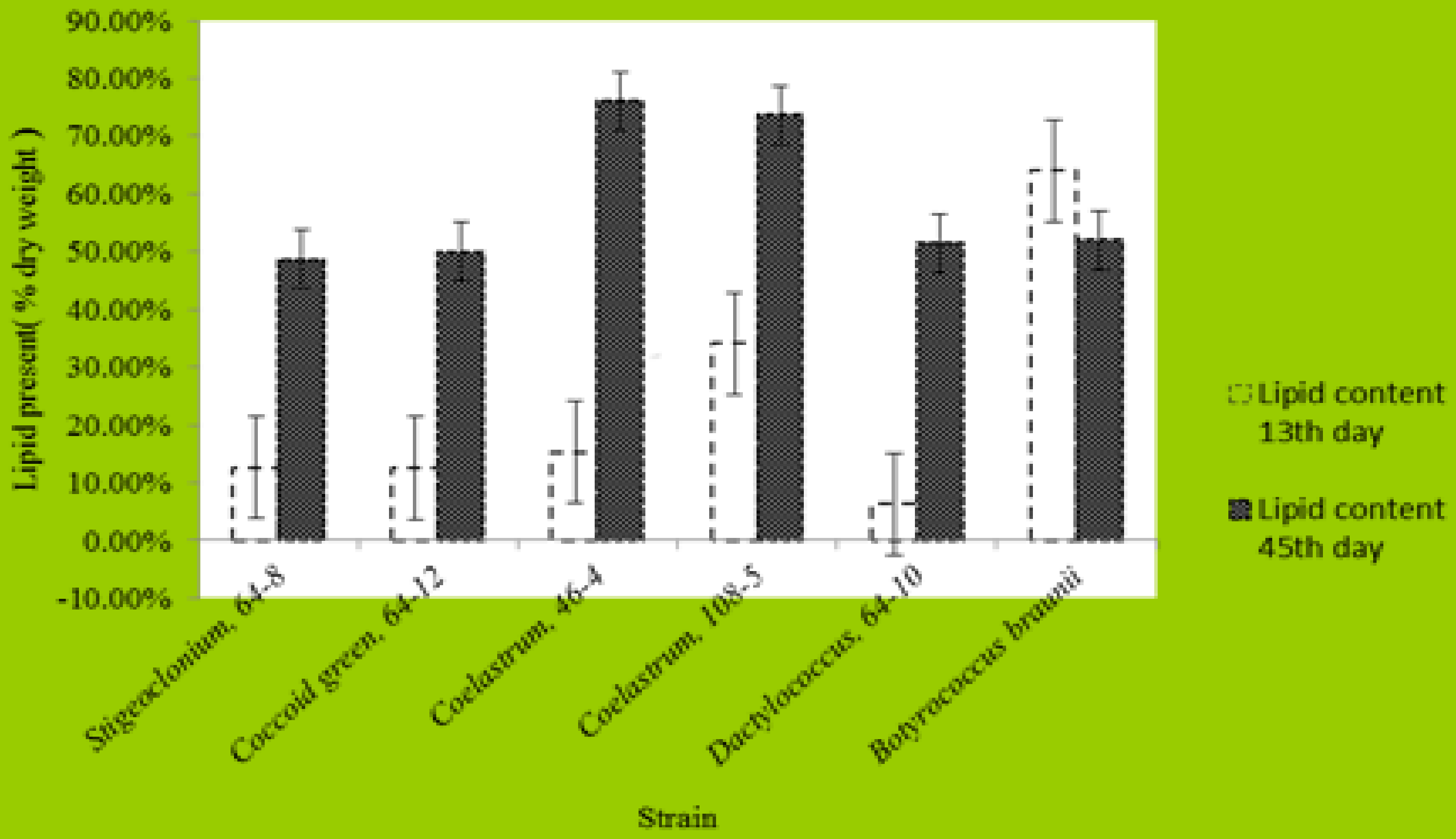
Strain	Lipid concentration (µg/100 µl culture) 13th day	Lipid concentration (µg/100 µl culture) 45th day
Pediastrum 81-6	9.39	25.81
Pediastrum 108-4	16.58	25.03
Coelastrum 108-5	12.98	**27.98**
Chlorella 2-4	8.17	12.36
Chlamydomonas EV 29	10.53	17.82
Coccoid green 56-5	0.92	24.02
Selanstrum 34-4	9.40	24.17
Chlorococcum 55-2	1.88	18.99
Chlorococcum 55-5	5.17	27.11
Dactylococcus 64-10	3.52	29.86
Coelastrum 46-4	7.68	38
Scenedesmus 103-4	1.59	22.65
Chlorococcum 45-3	6.69	25.73

Coccoid Green 64-12	8.17	36.44
Scenedesmus 66-1	12.36	18.11
Chlorococcum 5-1	14.71	26.88
Pediastrum 80-15	1.57	16.78
Chlorella 71-4	2.55	18.78
Scenedesmus 81-5	10.32	14.42
Prochloro 104-1a	1.02	16.01
Coccoid Green 81-7	0.50	21.07
Scenedesmus 79-1	1.84	26.92
Stigeoclonium 64-8	7.70	29.72
Coccoid Green 56-4	6.40	15.88
Coccoid green 103-6	14.10	20.96
Kircherniella 104-7	2.84	27.54
Botryococcus braunii (Control)	33.91	27.54

The promising strains

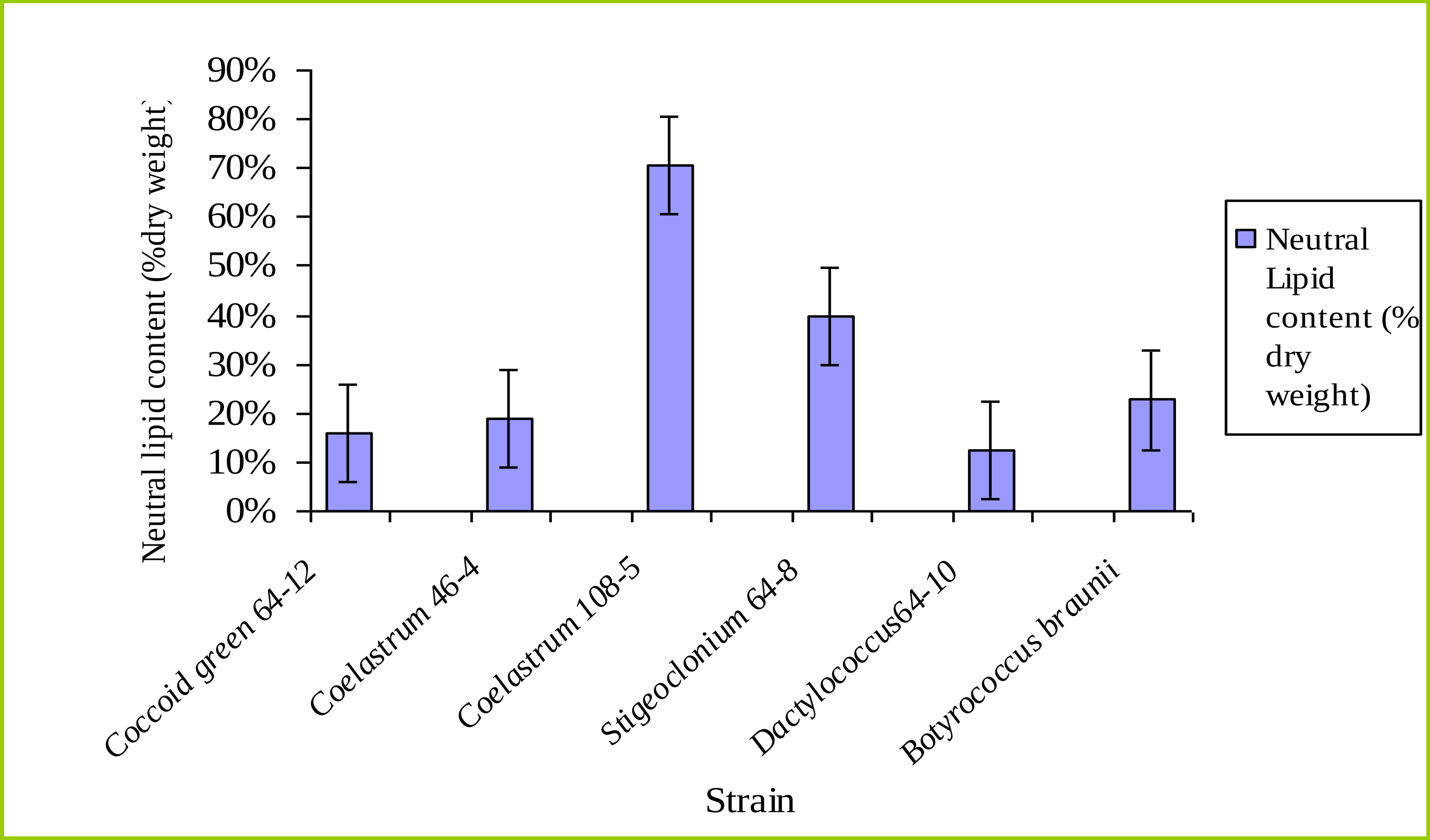
- *Coelastrum* 46-4, *Coccoid green* 64-12, *Stigeoclonium* 64-8 , *Dactylococcus* 64-10 and *Coelastrum* 108-5 were chosen based on their highest mean difference exhibited between the exponential and stationary phase.
- *Botyrococcus* showed a decrease in lipid content when it approached the stationary phase.
- *Chlorella* 2-4, *Chlamydomonas* EV-29, *Coccoid green* 103-6 and *Coccoid green* 56-4 did not show any significant accumulation of lipid between the two phases.

Lipid Content – Nile Red Method



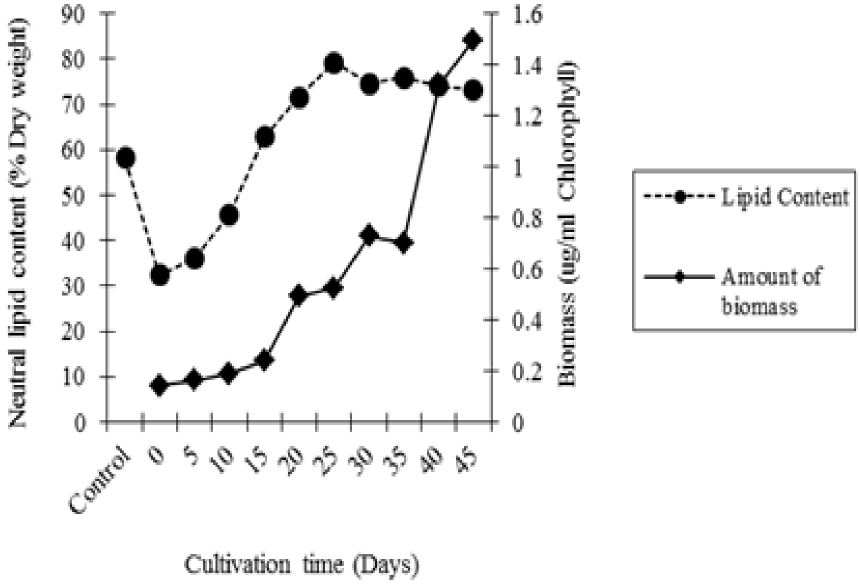
- Highest dry weight of biomass was observed in *Coelastrum* 46-4 (76%) followed by *Coelastrum* 108-5 (73.62%).

Lipid Content - Gravimetric Technique:

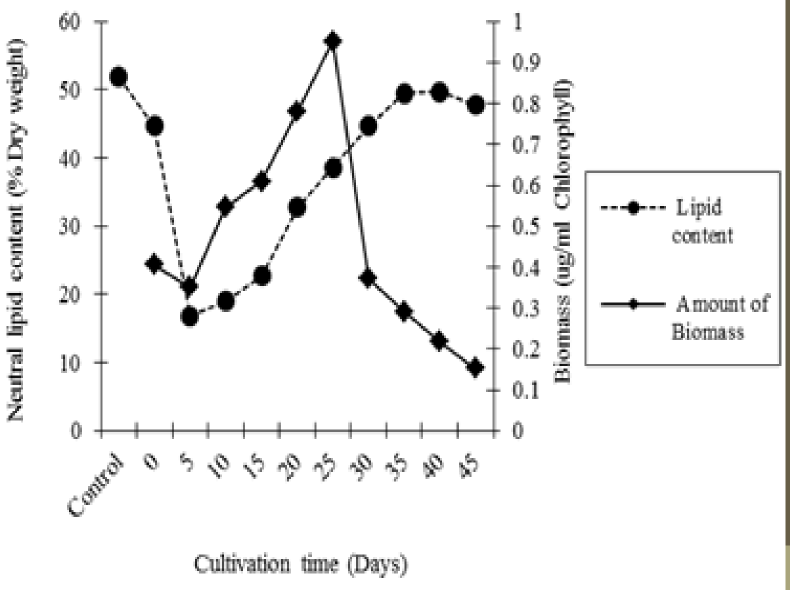


Lipid accumulation dependent on culture age

***Coccoid green* 64-12**



***Dactylococcus* 64-10**

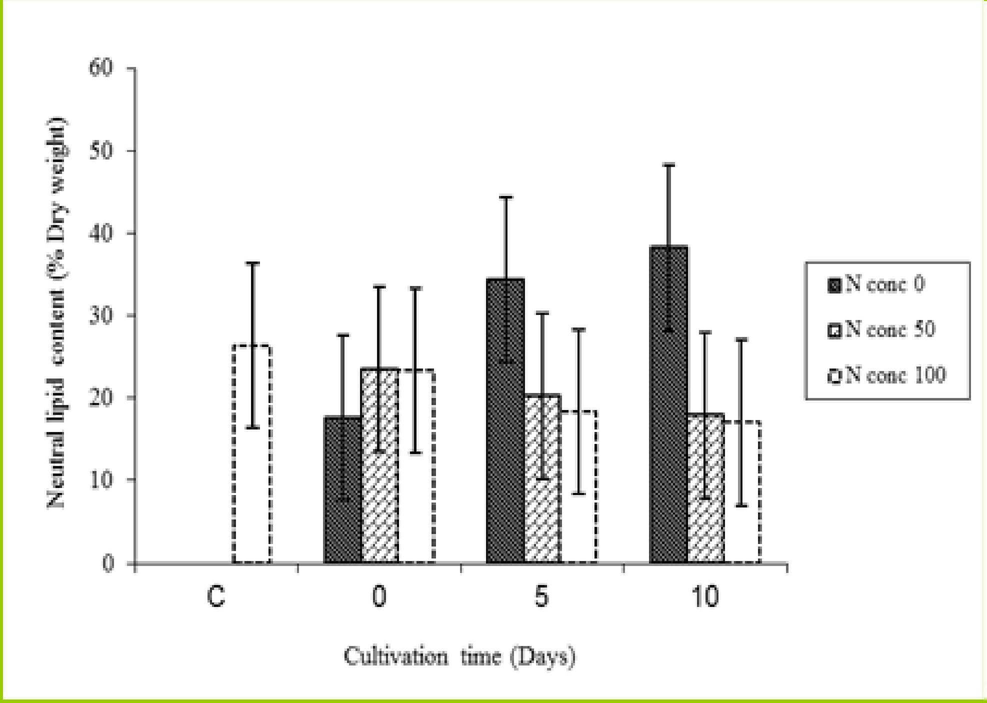


Biomass Yield and Lipid Accumulation

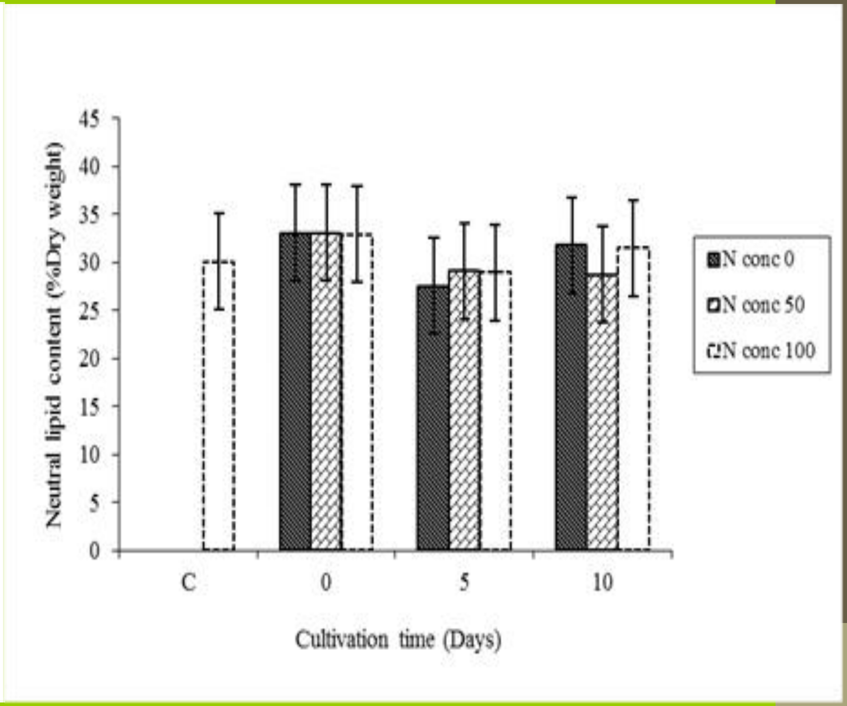
- *Positive correlation*
  - *Coccoid green* 64-12 (r= 0.834)
  - *Coelastrum* 108-5 (r = 0.703)
- No significant correlation
  - *Coelastrum* 46-4
  - *Dactylococcus* 64-10 (p>0.05).
- *Botyrococcus braunii*, showed a negative correlation of r=-0.861 .

Effect of nitrogen depletion

***Stigeoclonium* 64-8**



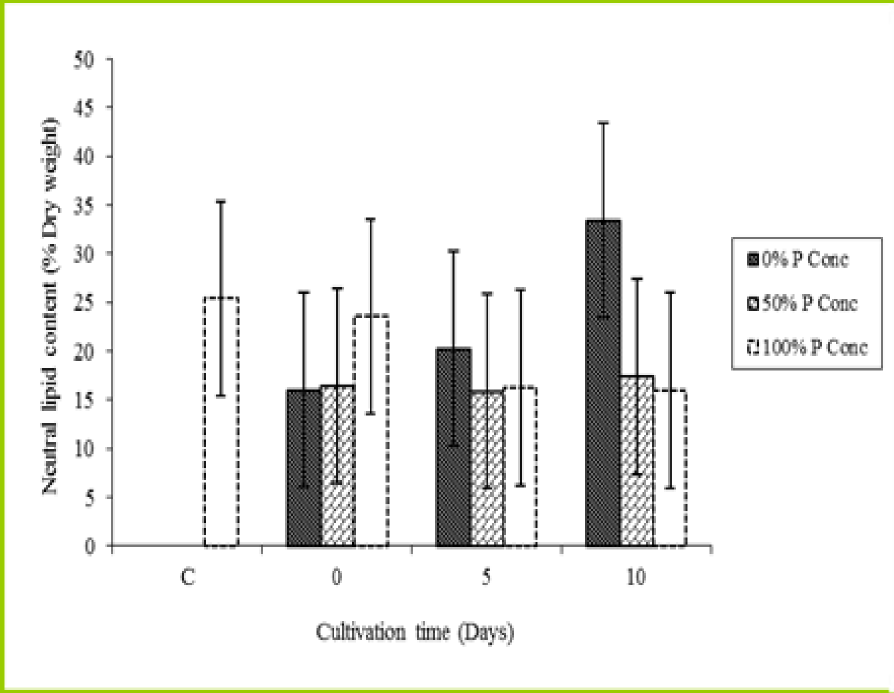
***Coelastrum* 108-5**



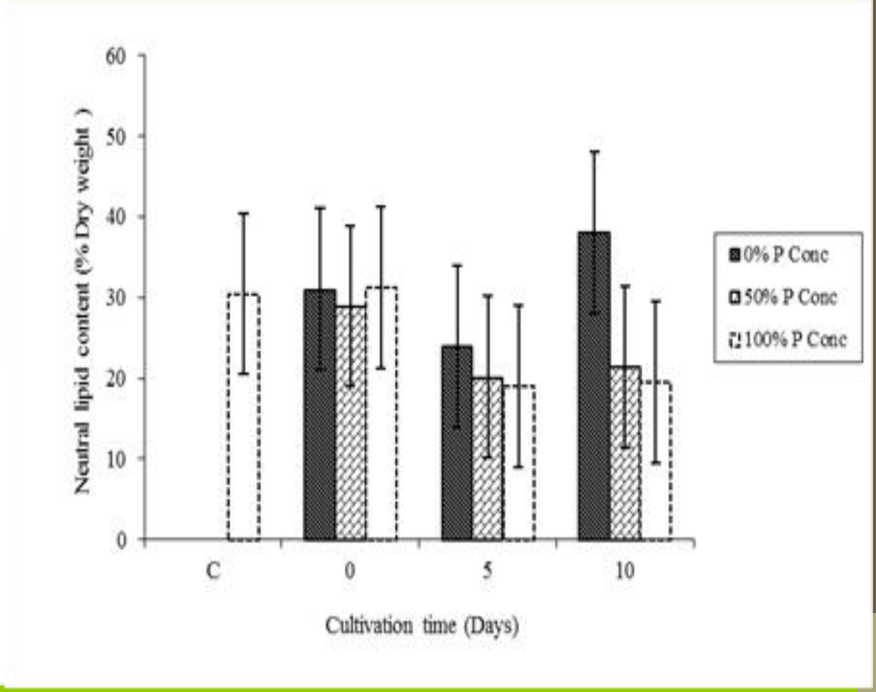
Strains responded: *Coccoid green* 64-12 and *Stigeoclonium* 64-8

Effect of Phosphorous depletion

***Stigeoclonium* 64-8**



Botyrococcus braunii



Strains responded: *Stigeoclonium* 64-8, *Coccoid green* 64-12 and *Coelastrum* 46-4.

Conclusions:

- *Coccoid green* 64-12, *Stigeoclonium* 64-8, and *Coelastrum* 108-5 were promising
- *Stigeoclonium* 64-8 and *Coccoid green* 64-12 were able to achieve high amount of lipid under nitrogen and phosphorous depleted conditions
- Each strain behaved differently under different environmental conditions

Thank You