CULTIVATION AND EXTRACTION OF MICROALGAL OIL BEING AS A RAW MATERIAL FOR SUSTAINABLE BIODIESEL PRODUCTION:
AUTOTROPHIC-HETEROTROPHIC-MIXOTROPHIC GROWTHS

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1. Introduction

Advantages of Microalgae for fuel production

- ✓ higher photosynthetic efficiency
- ✓ higher biomass production
- ✓ faster growth compare other energy crops
- ✓ higher lipid accumulation in cells

Biodiesel

- fatty acid methyl esters (FAMEs)
- biodegradable, renewable and non-toxic fuel.
- no net co_2 or sulfur
- emits less gaseous pollutants than normal diesel

2. Materials and methods

2.1 Microalgae strain

Chlorella vulgaris TISTR 8580

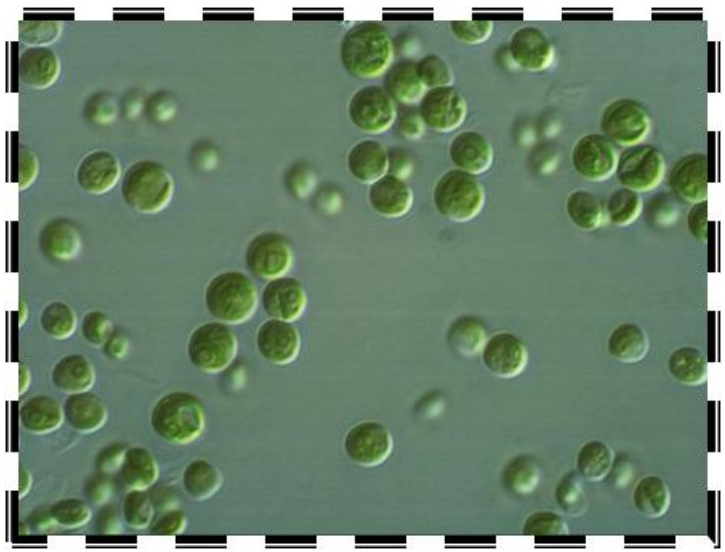
Division : Chlorophyta

Class : Chlorophyceae

Order : Chlorellales

Genus : *Chlorella*

Species : *Chlorella vulgaris*



- unicellular green microalgae
- fresh water
- non-motile

2.2 Cultivation

- The modified Bold's basal medium
- The initial 10^6 cells mL^{-1}
- pH 6.8 and temperature at 25°C.
- in 40 L photobioreactor containing 20 L medium
- Batch system with continuous aerated by using air pump

2.3 Cultivating conditions

1. Autotrophic

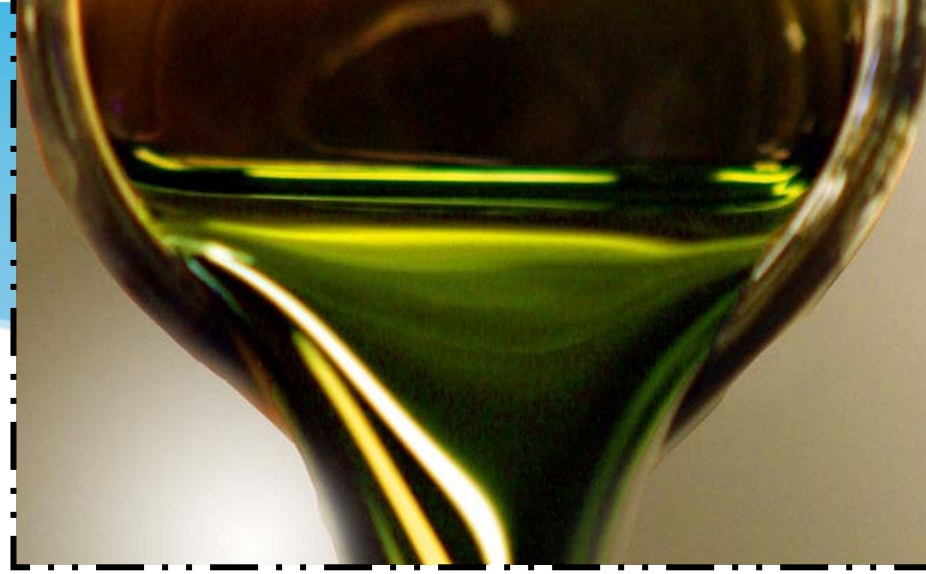
- light intensity 5000 lux
- interval periods
  16 hr light / 8 hr dark
- carbon sources $C0_2$
- nitrogen sources
  $NaNo_3$ 25 g/l

2. Heterotrophic

- No light (in the dark)
- carbon sources
  sucrose10 g/l
- nitrogen sources
  $NaNo_3$ 0.1 g/l

3. Mixotrophic

- light intensity 5000 lux
- interval periods
  16 hr light / 8 hr dark
- carbon sources
  sucrose10 g/l
- nitrogen sources
  $NaNo_3$ 0.1 g/l



3. Analytical techniques

- Cell growth ⇨ spectrophotometer A_{540} nm
- Cell numbers ⇨ microscope using haemacytometer
- Carbohydrate ⇨ Phenol sulfuric method
- Protein ⇨ Lowry's method
- Moisture ⇨ Weender analysis
- Ash ⇨ Weender analysis
- Lipid ⇨ hexane in a soxhlet extraction

4. Results and discussions

4.1 Autotrophic - Heterotrophic - Mixotrophic growths of *C. vulgaris*

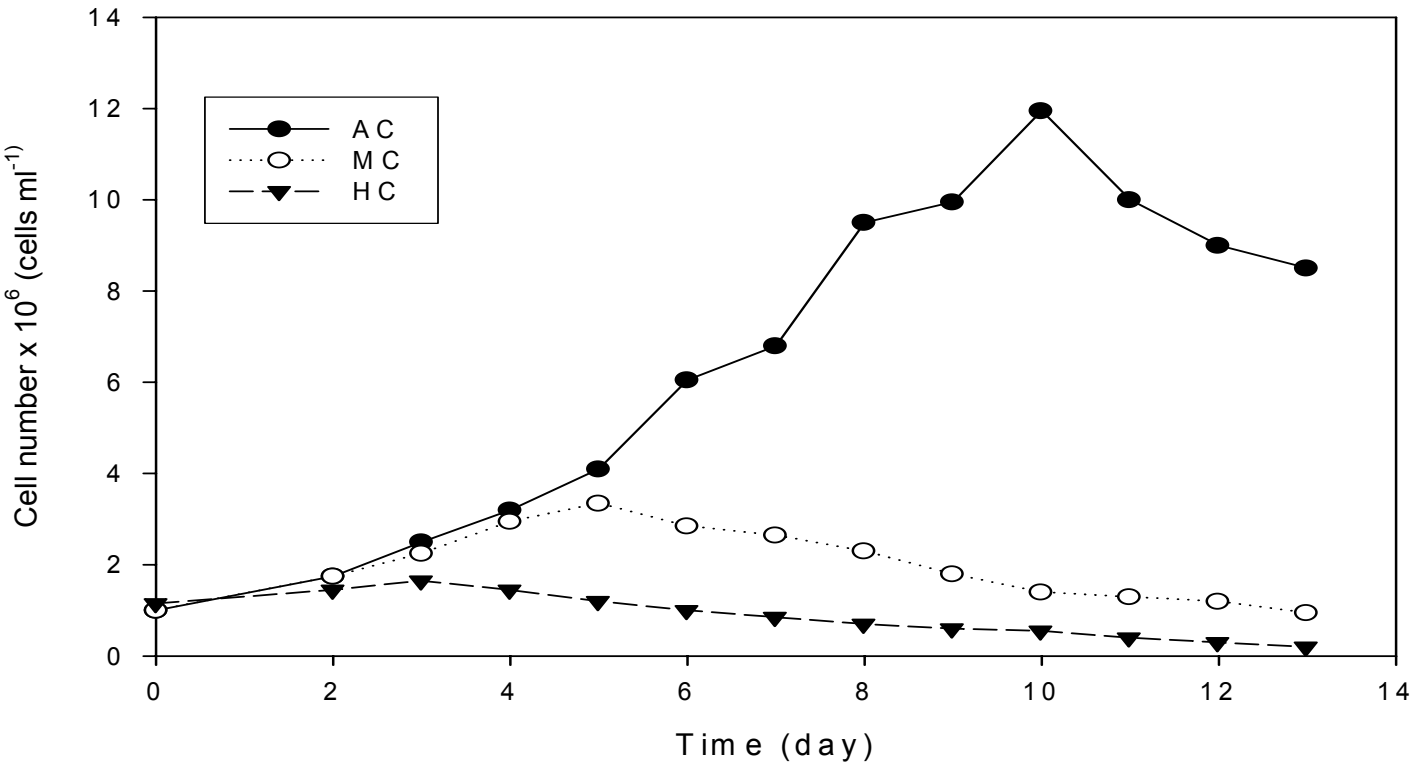
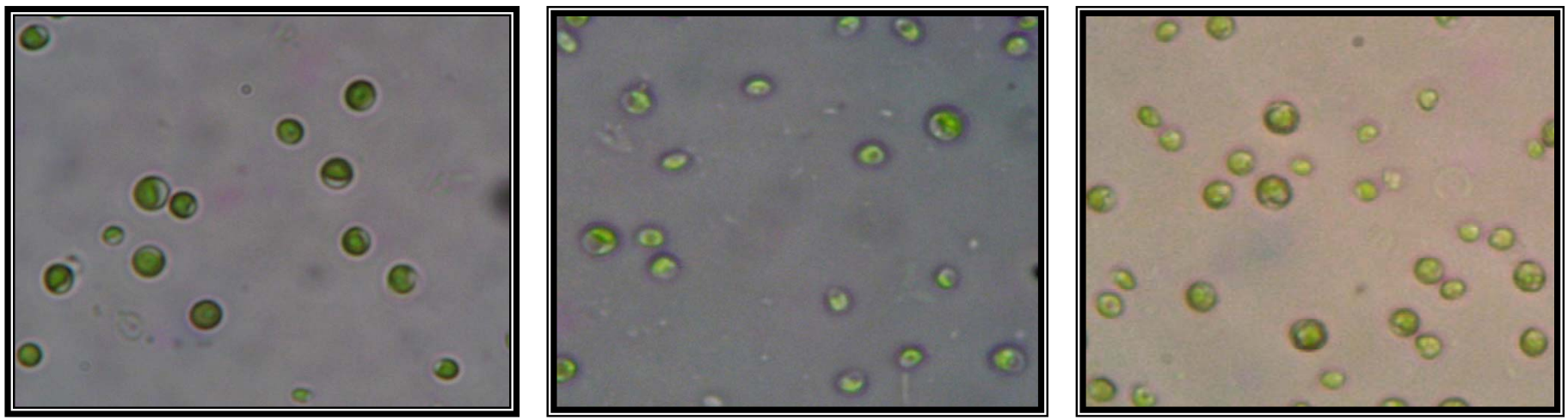


Fig. 1. Growth monitoring of *C. vulgaris* in cell number as functions of time
(● AC; Autotrophic cultivation ▼HC ; Heterotrophic cultivation, ○ MC ; Mixotrophic cultivation)

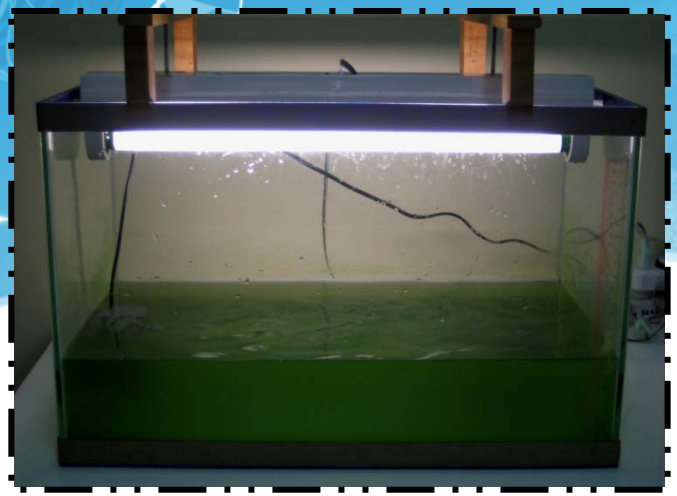
Table. 1 Growth parameters of *C. vulgaris* in AC, HC and MC conditions

Parameters	Autotrophic	Heterotrophic	Mixotrophic
Max. day growth (d)	10	3	5
Max. cell number (cells ml^{-1})	1.195×10^7	1.65×10^6	3.35×10^6
Max. dry weight (gL^{-1})	0.8014	0.4072	0.6217
Max. specific growth rate (μ_{max}, d^{-1})	0.3026	0.1800	0.2920



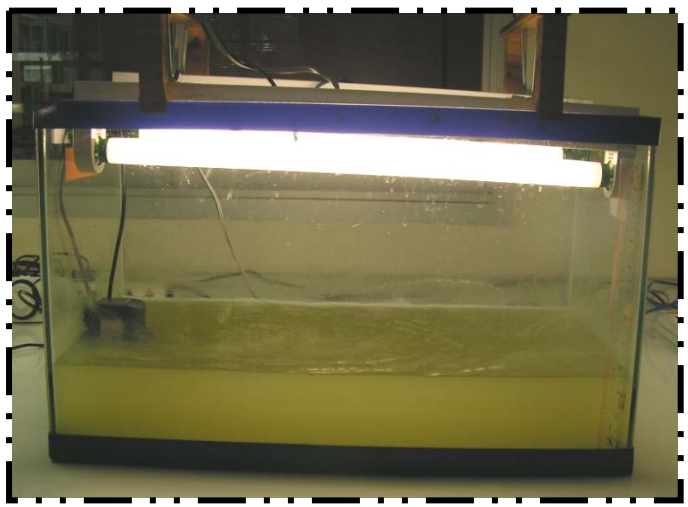
(a) (b) (c)

Fig. 2 Characteristics of *C. vulgaris* cells under microscope in cases of (a) autotrophic cultivation (b) heterotrophic cultivation and (c) mixotrophic cultivation, respectively.



(a)

(b)



(c)

Fig. 3 Growth of *C. vulgaris* in 20 L photo bioreactor (a) autotrophic cultivation (b) heterotrophic cultivation and (c) mixotrophic cultivation, respectively.

4.2 Characteristics of *C. vulgaris*

Carbohydrate

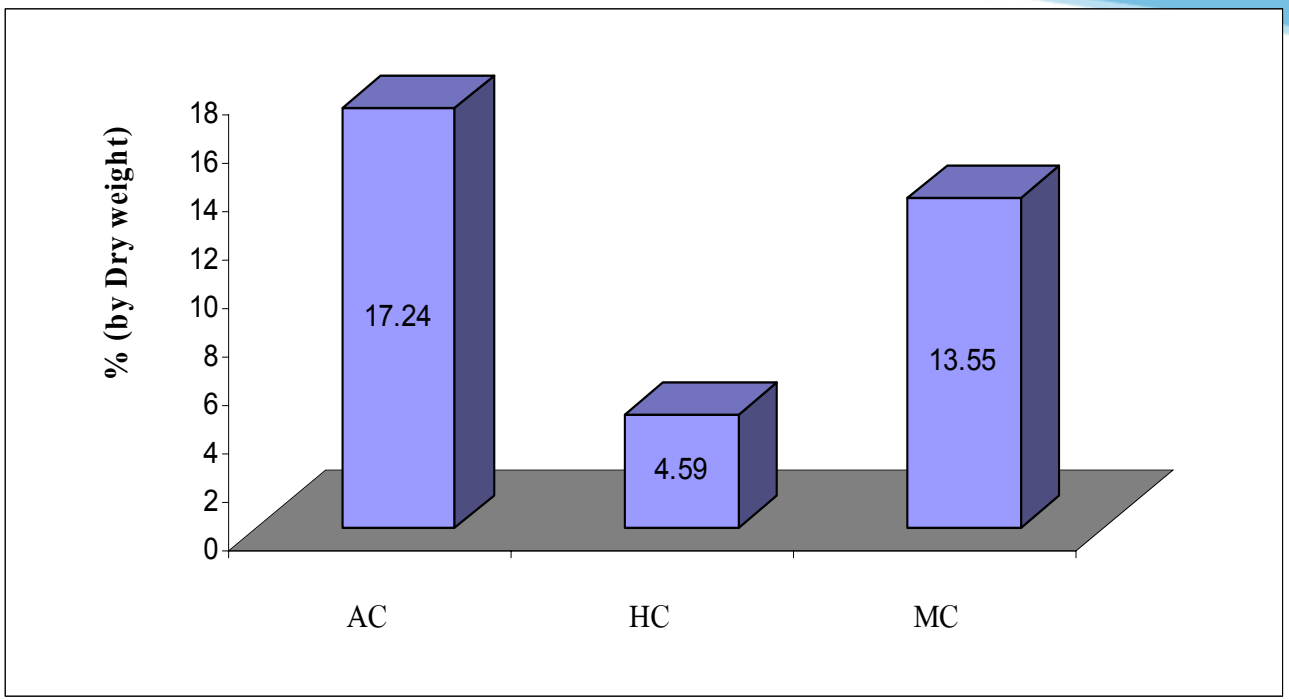


Fig. 4 the carbohydrate content founded in AC 17.24% HC 4.59% and MC 13.55%, respectively. (AC) autotrophic cultivation (HC) heterotrophic cultivation and (MC) mixotrophic cultivation.

Protein

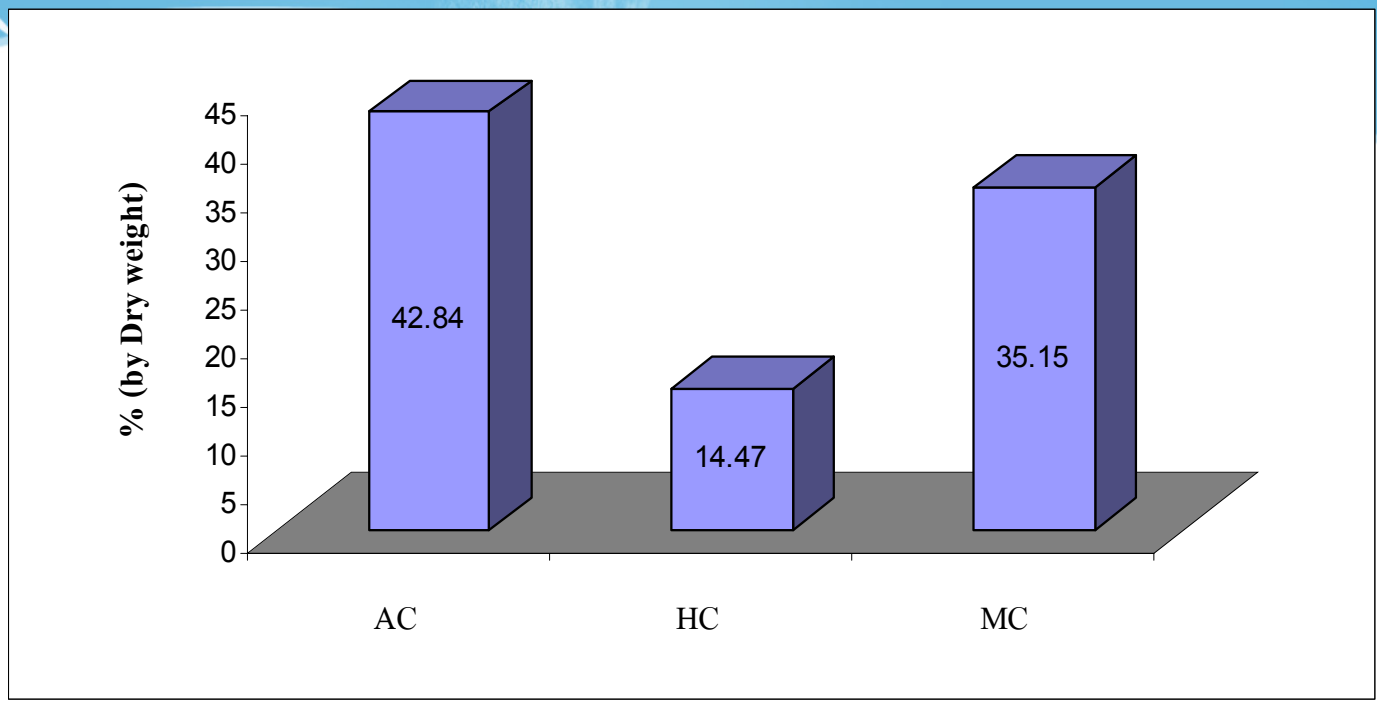


Fig. 5 the protein content founded in AC 42.84% HC 14.47% and MC 35.15%, respectively. (AC) autotrophic cultivation (HC) heterotrophic cultivation and (MC) mixotrophic cultivation.

Moisture

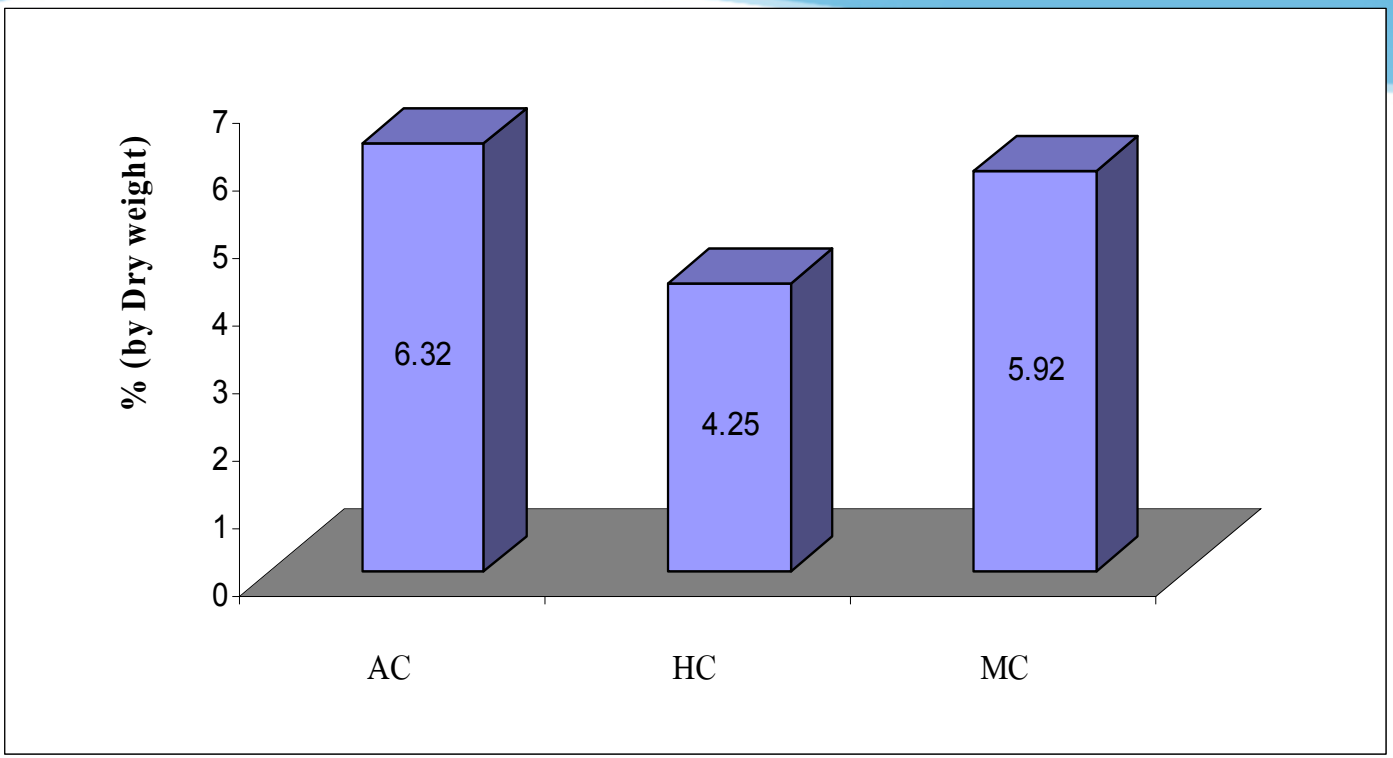


Fig. 6 the moisture content founded in AC 6.32% HC 4.25% and MC 5.92%, respectively. (AC) autotrophic cultivation (HC) heterotrophic cultivation and (MC) mixotrophic cultivation.

Ash

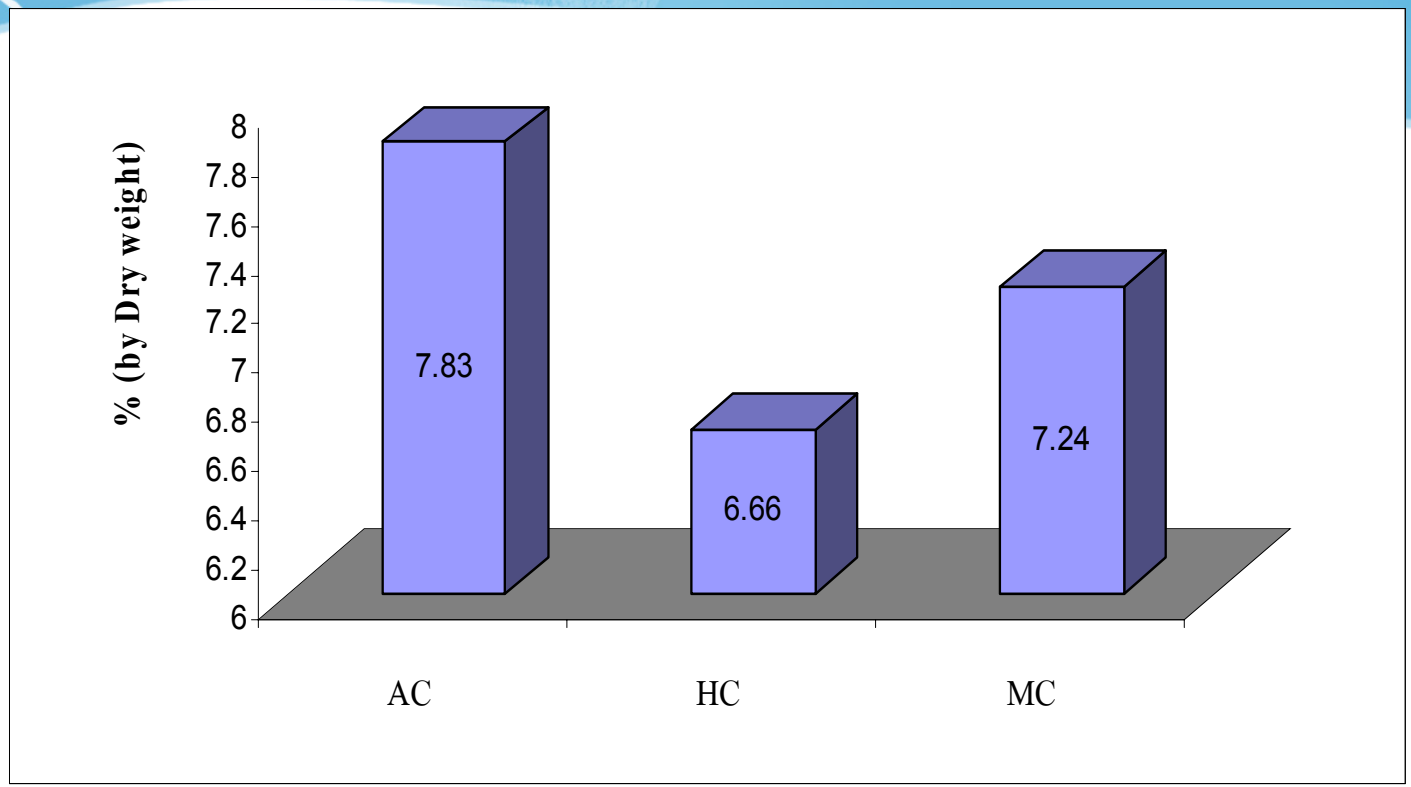


Fig. 7 the ash content founded in AC 7.83% HC 6.66% and MC 7.24%, respectively. (AC) autotrophic cultivation (HC) heterotrophic cultivation and (MC) mixotrophic cultivation.

Lipid

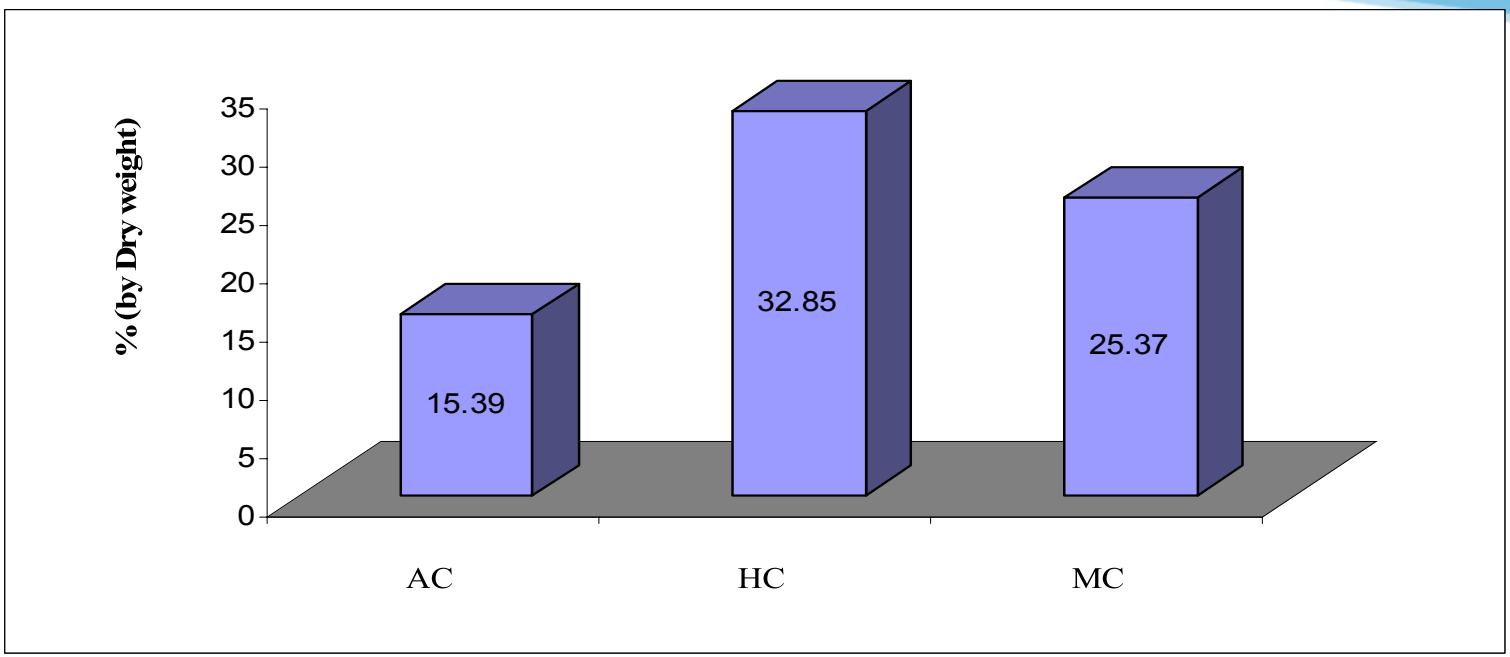


Fig. 8 the lipid content founded in AC 15.39% HC 32.85% and MC 25.37%, respectively. (AC) autotrophic cultivation (HC) heterotrophic cultivation and (MC) mixotrophic cultivation.

Soxhlet extraction

Algal oil + Hexane

Algal oil

Fig. 9 Lipid extraction

5. Conclusions

- **Heterotrophic and Mixotrophic growth of *C. vulgaris* results in not only the disappearance of chlorophyll in cells but also accumulation of high lipid content in cells.**

- **The heterotrophic cultivation provided for this algae strain can be reached to 32.85% by dry weight which was about 2 times that in autotrophic cells (15.39%)**

- **The algal cell from heterotrophic cultivation obtained showed in smaller form than others and also was difficult to control during cultivation.**

- **In further work, Mixotrophic cultivation was selected to produce microalgal oil for biodiesel production.**

6. Acknowledgements

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7. References

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