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OPTIMIZATION OF PROCESS VARIABLES FOR PRODUCTION OF BIO OIL FROM MIXED ALGAL CULTURE FROM TAMMILERU LAKE AND GODAVARI RIVER

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ABSTRACT

Optimization of process variables for the production of bio oil from mixed algal cultures collected from Godavari river and tammileru lake, Godavari Dist, A.P., was carried out using traditional OFAT (One Factor At a Time) method.

KEYWORDS: bio oil, mixed algal cultures, optimization, OFAT.

INTRODUCTION

In the development of recent years, most advances have been made in the specialized and financial improvement of algal biofuel production. In 2010, the "state of the art" advances used to create biodiesel from green growth included paddle-wheel driven pond development, centrifugation-based harvesting, biomass drying, and trans-esterification and resulted in fuel expenses of over $100/agge (Amanda et al., 2015). Exhaustion of non-renewable energy sources and raising costs has made research on biodiesel as the need of great importance. Biodiesel is a handled fuel got from the esterification and transesterification of free unsaturated fats and triglycerides, individually that happen actually in inexhaustible natural sources, for example, plant oils and animal fats. Also, the accessibility of the oil crops fill in as the hotspots for the biodiesel generation is restricted. Along these lines, it is important to discover new feedstock appropriate for biodiesel production, which does not deplete on the edible vegetable oil supply. One contrasting option to the regular oil yield is the green growth since they contain oil, reasonable for esterification/trans esterification response for the biodiesel generation (Renita et al., 2012). Algae are effective makers of natural oils, sequester carbon dioxide in this manner decreasing greenhouse gasses, and don't compromise a food stock or deplete soil supplements. Discovering high lipid delivering strains and choosing the suitable refined and handling conditions are basic to understand the potential and huge scale adoption of advanced algal biofuels. When put into stressful environments (e.g. nutrient starvation), algae may switch carbon allocation from reproduction to oil production. This oil from algae can be removed and transformed into biodiesel through a synthetic procedure called transesterification (Csavina et al., 2011). Bio-oil has a few natural preferences over non-renewable energy sources as a clean fuel. Bio-oils are CO2/GHG neutral. Therefore they can, produce carbondioxide credits. NoSOx emissions are generated, because plant biomass contains insignificant amounts of sulfur. Consequently, bio-oil would not be subjected to SOx charges. Bio-oil fills produce more than half lower NOx emanations than diesel oil in a gas turbine. Thusly, overhauling of bio-oil is important to give a fluid item that can be utilized as a fluid fuel or compound feedstocks in different applications. As of late, scientists and business people have centered their advantage, particularly on the algal biomass as the option feedstock for the generation of biofuels. Additionally, algal biomass has no opposition with farming sustenance and nourish generation (Shuvashish et al., 2015).

Production of biofuel using microalgae biomass appears to be a viable alternative. The oil productivity of many microalgae exceeds the best producing oil crops. While a number of bio-feed stocks are currently being experimented for biofuel production, algae have emerged as one of the most promising sources for biofuel production (Neeraja et al., 2016). In the present study the optimization of production of Bio oil from mixed algal culture was studied by OFAT method.

MATERIALS AND METHODS

Bio oil Production Media (BPM)

Initially Bio oil production by mixed Algal culture was studied using various production media reported earlier. Table 1&2 and Fig 1, show the composition of 3 different well known Bio oil Production Media (BPM) that were used for culture media optimization.

Optimization of process parameters by OFAT (One factor at a time) method

For theoptimization of process variables using OFAT method, Bio oil Production Medium 3 (BPM 3) was used to optimize various fermentation parameters like temperature, pH, agitation, inoculum concentration, substrates, additional carbon sources, nitrogen sources and surfactants in submerged cultures. The algal cultures was separated by centrifugation at 10,000 rpm for 10 min and the filtrate was considered as a crude Bio Oil and stored at -20°C for further use.

Biomass determination

The Algal cultures obtained by filtering the culture broth through Whatman filter paper No. 1 was dried at 80°C (Silva *et al*., 2005). Dry weight of the algal biomass was calculated and expressed as mg mLG^{1}. Values were the mean of three sets of experiments run simultaneously.

RESULTS AND DISCUSSION

Effect of substrates

The suitable substrate for optimum Bio oil production was analyzed by adding various oils as substrates to BPM 3 viz., Olive oil, Sunflower oil, Ground nut oil, Palm oil, Gingelly oil, Coconut oil and Cotton oil. From the results presented in Fig. 3, it was evident that olive oil is the best substrate for Bio oil induction by mixed culture.

Effect of temperature

The effect of temperature on enzyme production was studied byincubating the fungus at different temperatures from 10-60°C and it was found that the enzyme production was maximum at 30°C (Fig. 4).

Effect of the medium pH

The effect of pH on Bio oil production was studied by culturing the fungus in BPM 3 for 96 h with a pH range 4-10 and it was found that optimum pH for Bio oil production was pH 6. However, the organism is able to show optimum Bio oil activity over a broad range of pH (Fig. 5).

Effect of agitation

Agitation increases the metabolism as the organism is capable of utilizingoxygen very well. In the present study, effect of agitation on Bio oil production was studied by incubating the organism in a rotary shaker incubator at 0 (stationary) 100, 150 and 200 rpm. The results (Fig. 6), obtained shows that Bio oil production was maximum at 150 rpm.

Effect of inoculum concentration

The effect of inoculum concentration on Bio oilproduction was studied by incubating the organism in a production medium containing different concentrations (1-10%) of the inoculum and from the results (Fig. 7) it was found that a variation in inoculum concentration does not affect the enzyme production much.

Effect of additional carbon sources

To check whether carbon source in the form ofcarbohydrates affect the Bio oil production, various sugars viz., Glucose, Fructose, Galactose, Arabinose, Maltose and Sucrose at a concentration of 1% were added to LPM containing olive oil as primary substrate. The data obtained (Fig. 8) indicates that no sugar added as additional carbon sources enhanced the enzyme production compared to control.

Effect of organic and inorganic nitrogen sources

Effect of organic and inorganic nitrogensources on the Bio oil production was studied by replacing the nitrogen source of BPM 3 with various organic (1%) and inorganic nitrogen sources (1%) such as Peptone, Yeast extract, Malt extract, Beef extract, Ammonium sulphate, Ammonium nitrate, Ammonium dihydrogen phosphate, Ammonium chloride, Sodium nitrate etc., with olive oil as substrate. Though the enzyme production was also significantly enhanced with yeast extract, an organic nitrogen source but the production was maximum with ammonium sulphate, an inorganic nitrogen source (Fig. 9 a-b).

Effect of surfactants

Effect of various surfactants like SDS, PEG, Tween 20, Tween 80, Gumarabic and triton X100 on the Bio oil production was studied by adding them at a concentration of 0.5% to BPM 3 containing olive oil as substrate, ammonium sulphate as nitrogen source with pH 6 and the culture flasks were incubated at 30°C for 96 h at 150 rpm. The data from Fig. 10 shows that Bio oil production was maximum with gum Arabic as surfactant compared to control without surfactant.

Table. 1: Composition of modified Chu-10 growth medium (Safferman and Morris, 1964).

Chu-10 medium (Safferman and Morris,1964)		**Modified Chu-10 medium(used as a basal medium)**			
Micrionutrients	**Concentration (g L^{-1})**	**Macronutrients**	**g L^{-1}**	**Micrionutrients**	**mg L^{-1}**
$Ca(NO_3)_2.4H_2O$	0.232	$CaCl_2.2H_2O$	0.036	H_3BO_3	0.5
$MgSO_4$ $7H_2O$	0.025	$MgSO_4$ $7H_2O$	0.025	$ZnSO_4.7H_2O$	0.05
Na_2CO_3	0.02	Na_2CO_3	0.02	$MnCl_2.4H_2O$	0.5
$Na_2SiO_3.5H_2O$	0.044	$Na_2SiO_3.5H_2O$	0.044	$CuSO_4.5H_2O$	0.02
K_2HPO_4	0.01	K_2HPO_4	0.01	MoO3	0.01
Ferric Citrate	0.0035	Ferric Citrate	0.0035	$CoCl_2$	0.04
Citic acid	0.0035	Citic acid	0.0035		
		KNO_3	1.10		

Tables 2: Composition of, BG-11 medium (Rippka *et al.*, 1979)

S.No	Micrionutrients	Concentration ($g\ L^{-1}$)
1	$NaNO_3$	1.5
2	K_2HPO_4	0.04
3	$MgSO_4\ 7H_2O$	0.075
4	$CaCl_2\ .2H_2O$	0.036
5	Na_2CO_3	0.02
6	Citic acid	0.0006
7	Ferric ammonium citrate	0.0006
8	EDTA (Na_2Salt)	0.001
9	H_3BO_3	2.86
10	$MnCl_2\ .4H_2O$	1.81
11	$ZnSO_4\ .7H_2O$	0.22
12	$Na_2MoO_4\ .2H_2O$	0.39
13	$CuSO_4\ .\ 5H_2O$	0.079
14	$Co(NO_3)_2.6H_2O$	0.049

Table-3 BPM-3 .Knop medium

KNO_3	1000 mg/l
$Ca(NO_3)_2$	100 mg/l
K_2HPO_4	200 mg/l
$MgSO_4.7H_2O$	100 mg/l
$FeCl_3$	1 mg/l
Trace mix	1 ml /l
Distilled Water	1000 ml

The pH of the medium is 7.0.

Composition of Trace mix mg/ litre distilled water is

$Fecl_3$	5 mg/l
$CaCl_2.2H_2O$	26.5mg/l
$MnCl_2.4H_2O$	0.02 mg/l
$CoCl_2.6H_2O$	0.02 mg/l
$CuSo_4$	0.01 mg/l
$ZnSO_4.7H_20$	0.04 mg/l
$Na_2Mo\ O_4.2H_2O$	0.02 mg/l
Na_2EDTA	6.5 mg/l

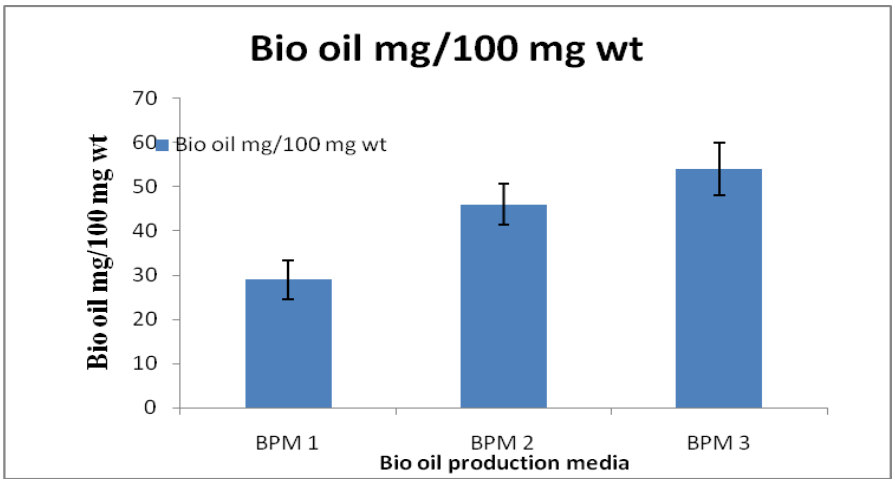


Fig. 1: Effect of different Bio oil production media on Bio oil production by mixed algal culture cultivated in Bio oil production media viz; BPM1, BPM2 & BPM3 are incubated at 30°C for 4 days at 150 rpm and oil produced was estimated.

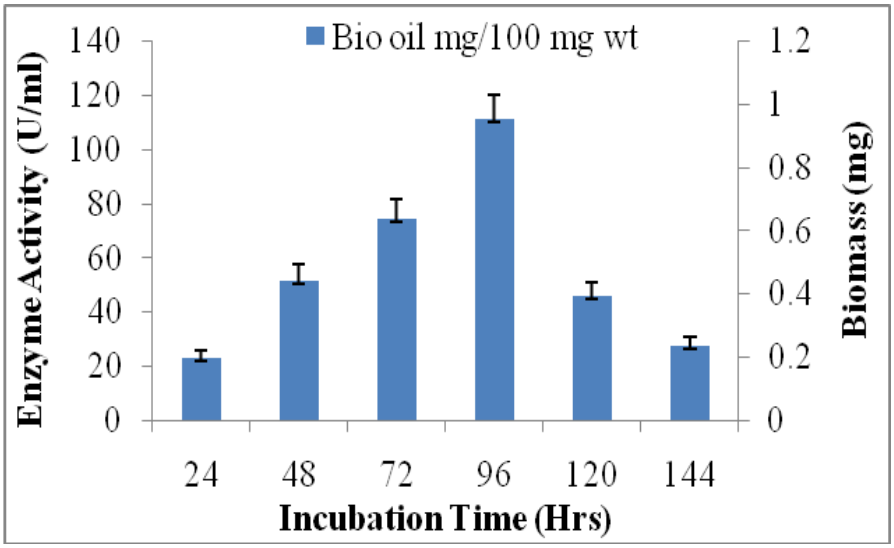


Fig. 2: Effect of incubation time on Bio oil production from mixed algal cultures suspension was inoculated into BPM 3 and incubated for different time periods viz., 24, 48, 72, 96, 120 and 144 at 30°C followed by enzyme estimation

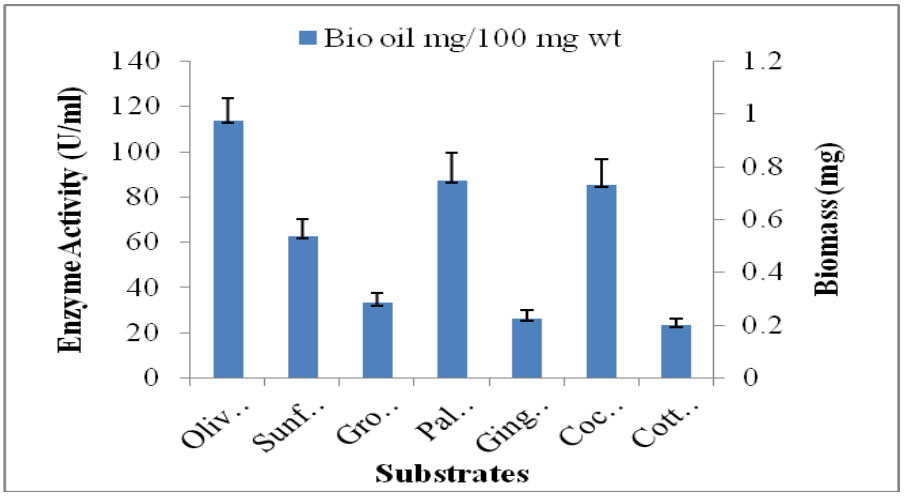


Fig. 3: Effect of different oil substrates on Bio oil production from mixed algal cultures was inoculated into BPM 3 with 1.5% each of various substrates viz., olive oil, sunflower oil, ground nut oil, palm oil, gingerly oil, coconut oil and cotton oil and incubated at 30°C for 96 h at 150 rpm and enzyme activity was estimated.

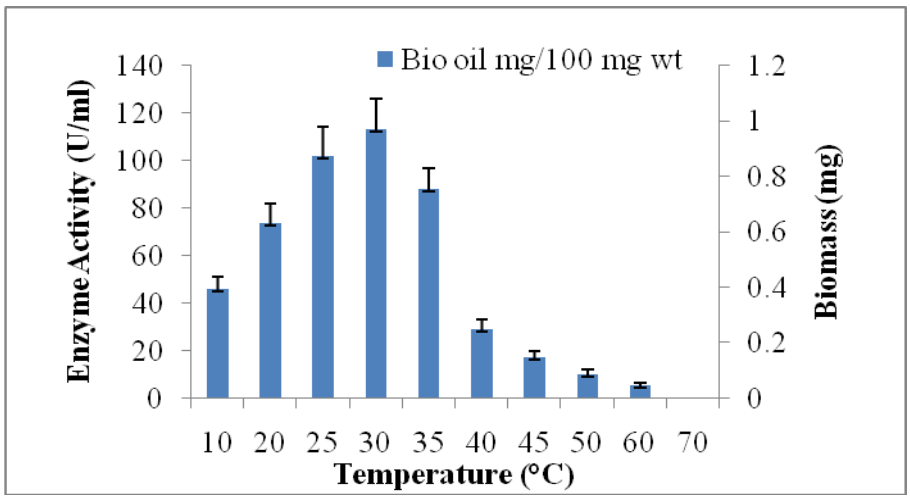


Fig. 4: Effect of incubation temperature on Bio oil production from mixed algal cultures was inoculated into BPM 3 containing olive oil as substrate and incubated at different temperatures from 10-70°C for 96 h at 150 rpm and enzyme activity was estimated

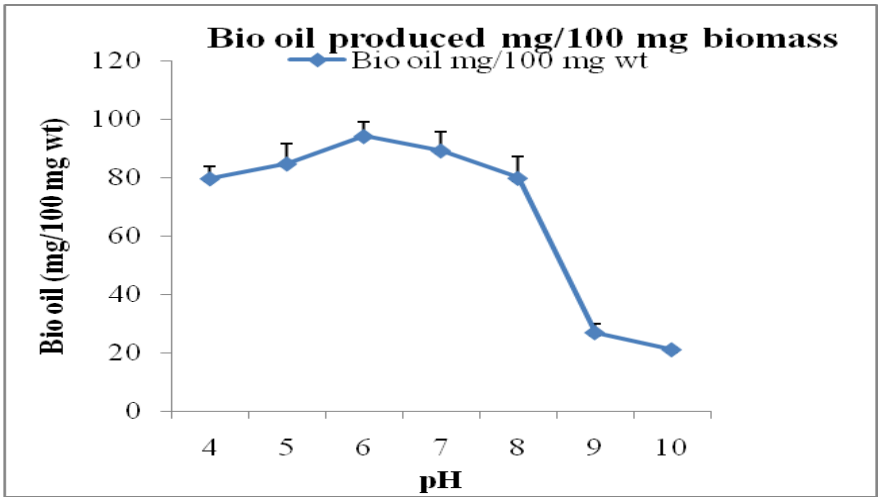


Fig. 5: Optimization of pH for Bio oil production from mixed algal cultures was inoculated into BPM 3 containing olive oil as substrate with varying pH ranging from 4-10 and incubated at 30°C for 96 h at 150 rpm and enzyme activity was estimated.

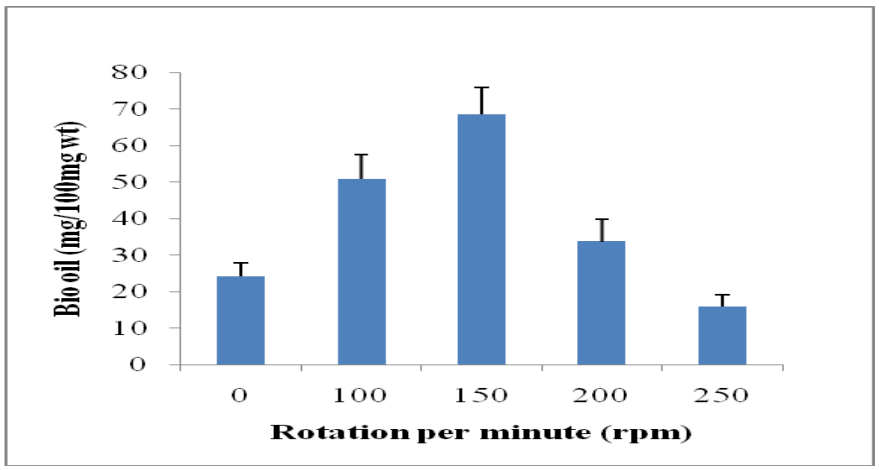


Fig. 6: Effect of agitation on Bio oil production from mixed algal cultureswas inoculated into LPM 3 of pH 6 containing olive oil as substrate incubated both under stationary and shake flask conditions (100, 150, 200 and 250 rpm) at 30°C for 96 h and enzyme activity was estimated.

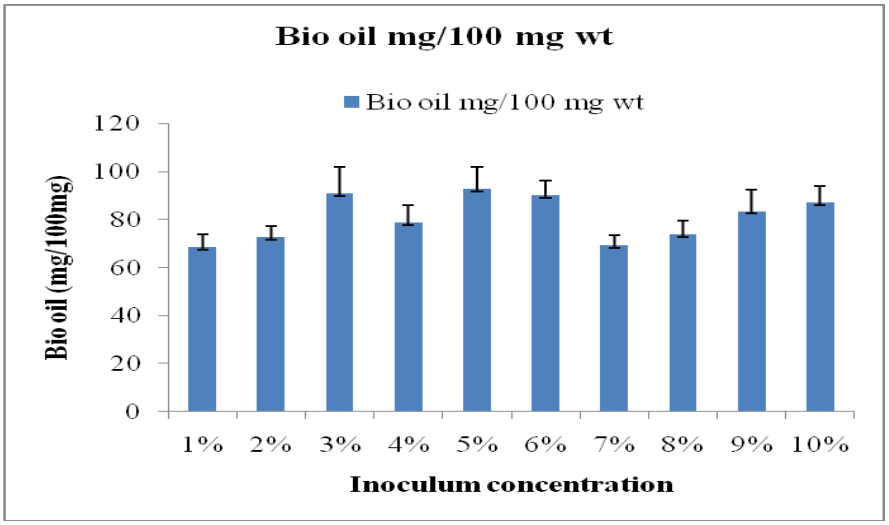


Fig. 7: Effect of inoculum concentration on Bio oil production from mixed algal cultures containing different concentrations of inoculum (1-10%) was inoculated into BPM 3 of pH 6, containing olive oil as substrate and incubated at 30°C for 96 h and enzyme activity was estimated.

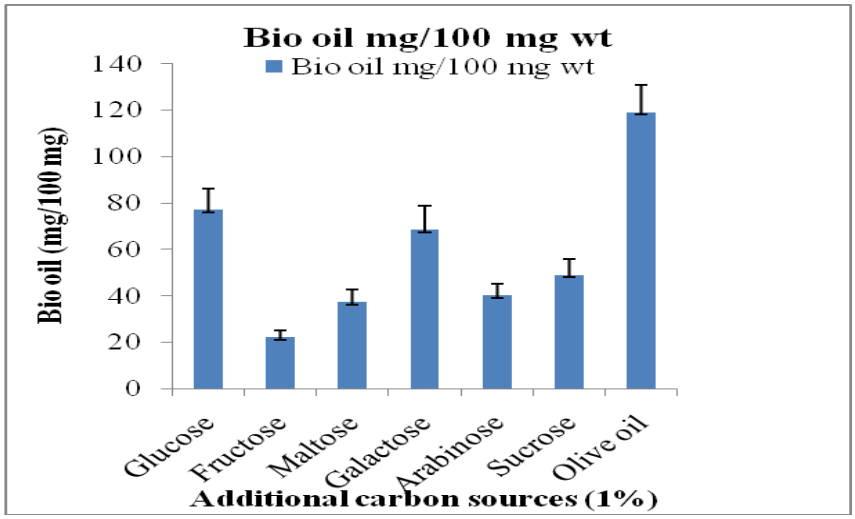
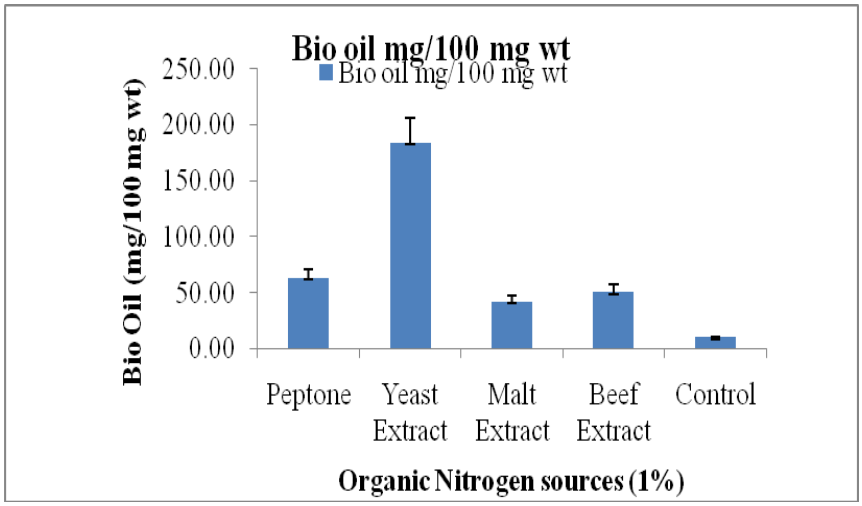


Fig. 8: Effect of different sugars as additional carbon source on Bio oil production-Mixed algal culture was inoculated into BPM 3 of pH 6 containing olive oil and 1% each of various additional carbon sources Viz., Glucose, Fructose, Galactose, Arabinose, Maltose and Sucrose and incubated at 30°C for 96 h and enzyme activity was estimated



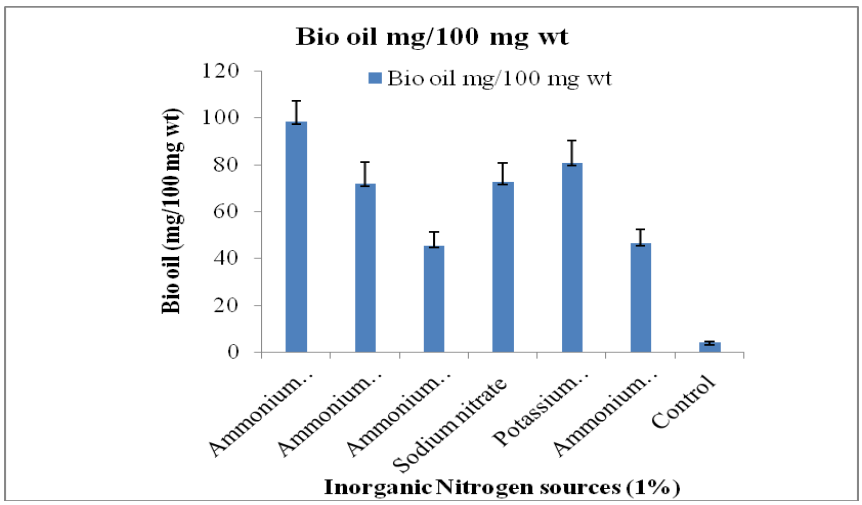


Fig. 9(a-b): Effect of organic and inorganic nitrogen sources on Bio oil production from mixed algal culture was inoculated into BPM 3 of pH 6 containing olive oil as substrate with 1% each of various organic and inorganic nitrogen sources viz., peptone, yeast extract, malt extract, beef extract, ammonium sulphate, ammonium nitrate, ammonium di hydrogen phosphate, ammonium chloride, sodium nitrate and incubated at 30°C for 96 h and enzyme activity was estimated

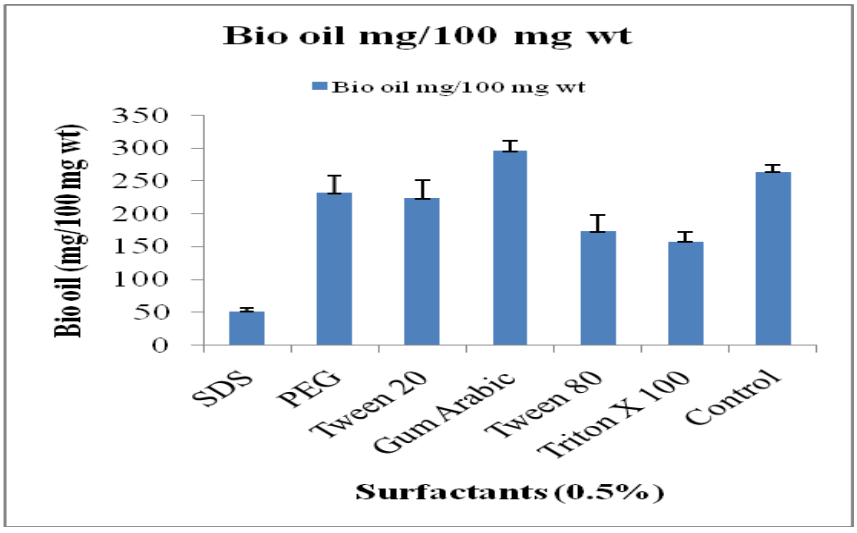


Fig. 10: Effect of surfactants on Bio oil production from mixed algal culture was inoculated into BPM 3 of pH 6, olive oil as substrate and ammonium nitrate as nitrogen source with 0.5% each of various surfactants viz., SDS, PEG, Tween 20, Tween 80, Gum Arabic and Triton X100 and incubated at 30°C for 96 h and enzyme activity was estimated.

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