The Pharma Innovation Journal 2022; 11(9): 51-55

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(9): 51-55

www.thepharmajournal.com
Received: 16-06-2022
Accepted: 27-08-2022

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Collection and screening of microalgae for growth and lipid accumulation

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Abstract
Microalgae are considered as potential feed stocks for biofuel production and are gaining much attention as a promising source for lipid production. The present study was aimed at the collection as well as screening of microalgae for their ability to accumulate lipids in their cells. Six microalgal water samples were collected from different locations of Haryana. The samples were analyzed for pH, EC (Electrical conductivity), salinity and turbidity by using the standard procedures. The pH of different algal water samples varied from 7.72 to 8.53 and electrical conductivity from 0.48 to 2.20 dSm^{-1}. Salinity was analyzed by determining the anions and Cations: CO_3^{2-}, Cl^-, Ca^{2+}, Mg^{2+} and HCO_3. The turbidity of algal samples varied from 38.5 to 75.0%. Enrichment of algal samples was carried out in Erlenmeyer conical flasks containing BG-11 broth using various concentration of dairy waste (whey). Growth of algal samples was determined by measuring optical density at 660 nm at regular time interval. The maximum biomass was produced by isolate KMA-1 i.e. 4.85 g/L in BG-11 medium supplemented with 30% whey. Fluorescence Microscopy that was used to screen the microalgal isolates for the presence of lipids indicated maximum lipid in the isolate KMA-1 on the basis of yellow fluorescence.

Keywords: Microalgae, biofuel, fluorescence, turbidity

Introduction
Continuous increase in world population, urbanization and industrialization are the prominent factors which contribute to fossil fuel depletion and raise energy demand. (Li *et al*., 2013). Energy security, increased oil prices, exhaustion of natural resources and climatic changes due to accumulation of harmful gases produced by burning of fossil fuels; are the major challenges for today's society (Sharma *et al*., 2019; Daroch *et al*., 2013; Chi *et al*., 2019) [20, 8, 4]. The energy crisis which has resulted due to depletion in fuel supplies has led researchers to focus on alternative renewable energy resources such as biofuels (Rawat *et al*., 2011; Rafiee and Khalilpour, 2019) [18, 17]. There are different generations of biofuels based on the substrate used for their production. A variety of feed stocks can be used for biofuel production such as molasses, corn, sugar beet, potato, wheat straw, paddy straw etc. But recent researches implicated to find out renewable energy not only from land plants or lignocellulosic biomass but also from the aquatic systems. In this context, oleaginous microalgae have received much attention for the production of renewable energy (Olguín, 2012; Mathimani, 2018) [16, 15]. Microalgae are microscopic unicellular organisms capable to harvest and convert solar energy to chemical energy via photosynthesis. Photosynthetic efficiency of microalgae is higher as compared to other oil crops like mustard, soya bean, sunflower etc. and these can accumulate higher quantity of lipids (Chisti *et al*., 2006; Chisti *et al*., 2007; Hu *et al*., 2008; Demirba *et al*., 2011) [6, 7, 13, 10]. These contain various bioactive compounds that can be harnessed for commercial use such as beta-carotene, astaxanthin, lectins, glycerol etc. Microalgae have long been recognized as promising substrate for renewable energy production because of their high oil content and rapid biomass production. Oil content of microalgae varies between 20-40% on dry weight basis whereas some microalgal strains contain up to 70% oil under certain conditions (Hidalgo *et al*., 2016) [12]. They can grow in a wide variety of climate and water conditions; can utilize and sequester CO_2 from many sources. These can be processed into a broad spectrum of products including biodiesel through trans-esterification of lipids, bioethanol through fermentation of algal biomass, biogas production via anaerobic digestion, bio-oil and bio-crude production via thermochemical conversion along with high protein animal feed. The lipid content of microalgae plays a very significant role for its use to produce biofuel and the concentration of lipid in microalgal biomass depends upon several factors such

as temperature, pH, nutrients etc. Lipid content of microalgal biomass can be determined using Nile Red staining technique which generally examines the non-polar lipids, it enables qualitative determination of lipids in the cells (Chen, 2011) [2]. Growth of microalgae can also be increased using industrial waste water such as dairy waste. Dairy waste water acts as a good supplement for the growth of algae along with BG-11 medium (Girard *et al*., 2014) [11]. Algae may offer a cost-effective and environmentally sustainable way of utilizing the nutrients. Therefore, the present study would be an effort in the direction of screening the efficient lipid producing strains of microalgae.

Materials and Methods

Collection of Microalgae

Random sampling method was applied for algal collection. The microalgal water samples used in the study were collected from ponds of different locations *viz*., Hisar, Kurukshetra, Ladwa and Pehowa.

Analysis of algal water samples

The samples were analyzed for pH, EC (Electrical conductivity), salinity and turbidity by using the standard procedures. Turbidity was measured by using the method of Chesnin and Yein (1950) [3].

Enrichment of Microalgae and Culture conditions

Enrichment of algal samples was carried out in Erlenmeyer conical flasks containing BG-11 broth. For enrichment ten ml of each algal sample was inoculated to the flasks separately and the flasks were incubated at 23-25 ºC, 50-60% humidity and 16:8 hours period of light: dark. The samples were incubated for a period of 3 weeks. The growth of algal isolates was determined spectrophotometrically at 660 nm at regular time interval.

Growth of Microalgal isolates

Algal isolates were grown on BG-11 medium by spread plate method and these were purified by streaking on plates containing BG-11 medium.

Screening of lipid producing Microalgae

Microalgae were further screened for presence of lipids by fluorescence microscopy using Nile Red staining.

Results and Discussion

The water samples collected from various locations and kept in Erlenmeyer flasks (Fig 1). These were analyzed for pH, EC (Electrical conductivity), salinity and turbidity by using the standard procedures. The pH of different algal water samples varied from 7.72 to 8.53 and electrical conductivity from 0.48 to 2.20 dSm^{-1}. Salinity was analyzed by determining the anions and Cations: CO_3^{2-}, Cl^-, Ca^{2+}, Mg^{2+} and HCO_3. Carbonate ions were absent in the microalgal water samples. Chloride ions ranged from 2.5 to 7.0 meq. Calcium and magnesium ions ranged from 2.0 to 13.5 meq and 5.0 to 40.0 meq respectively. Bicarbonates ranged from 3.5 to 20.0 meq. The turbidity of algal samples varied from 38.5 to 75.0% (Table 1).

Table 1: PH, Electrical conductivity and salinity analysis of microalgal water samples

Location		Samples	pH	Turbidity (%)	EC (dSm^{-1})	Salinity				
						CO_3^{2-}	HCO_3^{2-}	Cl^-	Ca^{2+}	Mg^{2+}
Kurukshetra	Bibipur	KMA 1	8.53	64.0	2.20	Nil	20.0	06.0	04.0	11.0
	Jyotisar	KMA 2	8.20	75.0	1.24	Nil	08.0	06.0	03.0	08.0
	Sura (Ladwa)	KMA 3	8.11	67.6	1.20	Nil	09.0	06.0	03.5	10.0
Pehowa	Jakhwala	PMA 1	7.72	59.0	2.10	Nil	04.0	02.5	13.5	40.0
Narwana	Gurthali	NMA 1	7.90	38.5	0.48	Nil	03.5	02.0	02.0	05.0
Hisar	Dahima	HMA 1	7.86	63.8	1.15	Nil	06.0	07.0	03.0	08.0

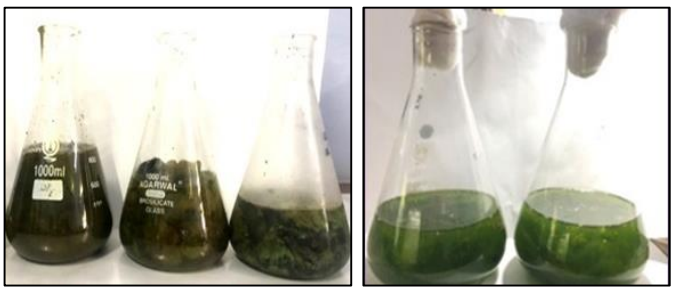

Fig 1: Microalgal water samples

Fig 2: Enrichment of microalgal samples

Enrichment of algal samples was done using BG-11 broth (Fig 2). Algal samples were then transferred to plates containing BG-11 medium, purified by streaking on the same medium (Fig 3) and microscopically examined (Fig 4).

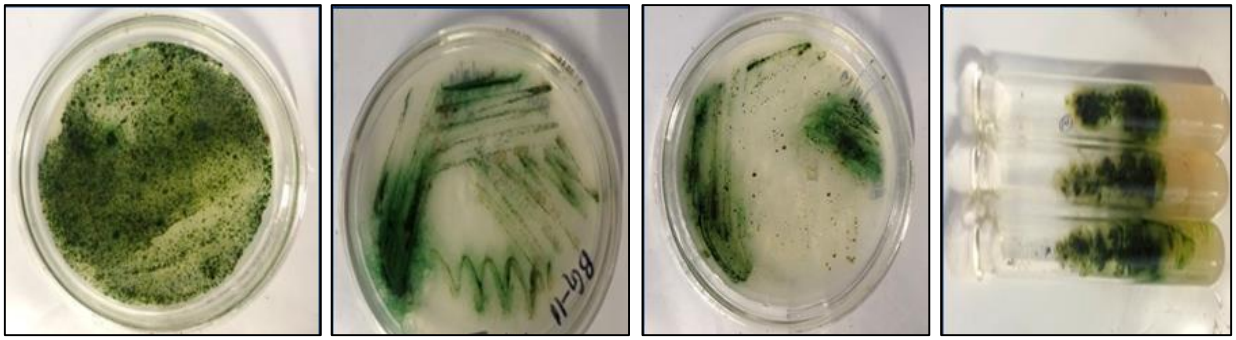

Fig 3: Isolation of microalgal strains and maintenance on slants

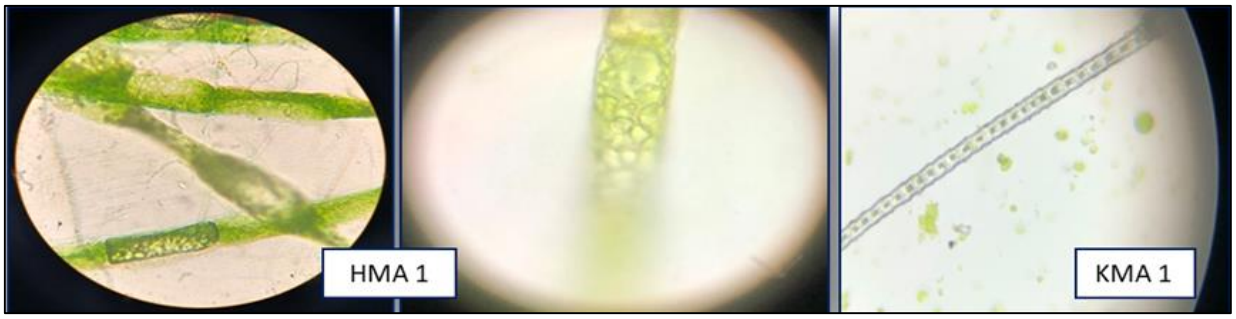

Fig 4: Microscopic view of algal isolates

Growth of algal samples was determined by measuring optical density at 660 nm at regular time interval. The broth was mixed thoroughly to make the sample homogenous before monitoring the optical density. All the isolates showed increase in growth up to 3 weeks (Fig 5). The maximum increase in O.D was observed in isolate KMA-1 *i.e.* from 0.059 to 0.241 (Table 2).

Table 2: Optical density of microalgal samples at different time interval

Isolates	O.D. at 660nm		
	1st week	2nd week	3rd week
KMA 1	0.059	0.137	0.241
KMA 2	0.041	0.109	0.136
KMA 3	0.046	0.125	0.185
PMA 1	0.037	0.098	0.127
NMA 1	0.049	0.132	0.199
HMA 1	0.056	0.129	0.211

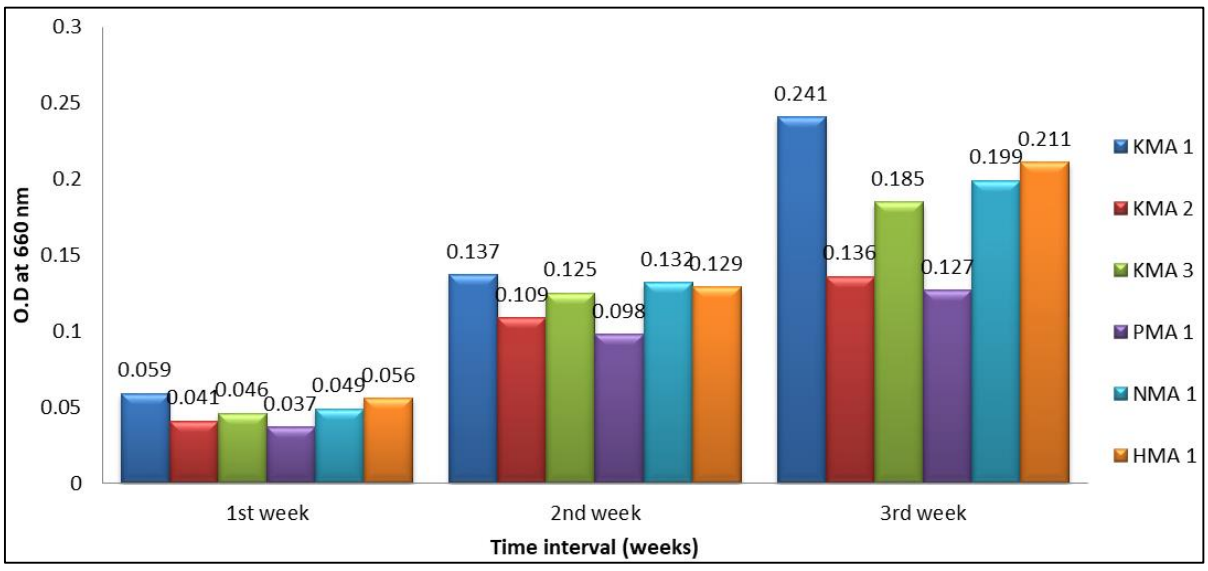

Fig 5: Growth curve of microalgal isolates

A number of processes exist for collection of algal biomass like chemical, mechanical, physical and biological methods (Uduman *et al*., 2010; Christenson and Sims, 2011) [21, 7]. The algal biomass was harvested after 21 days using sedimentation and centrifugation techniques. The wet biomass of different isolates was collected and kept for drying at 60-80 ºC till it reached its constant weight (Fig 6).

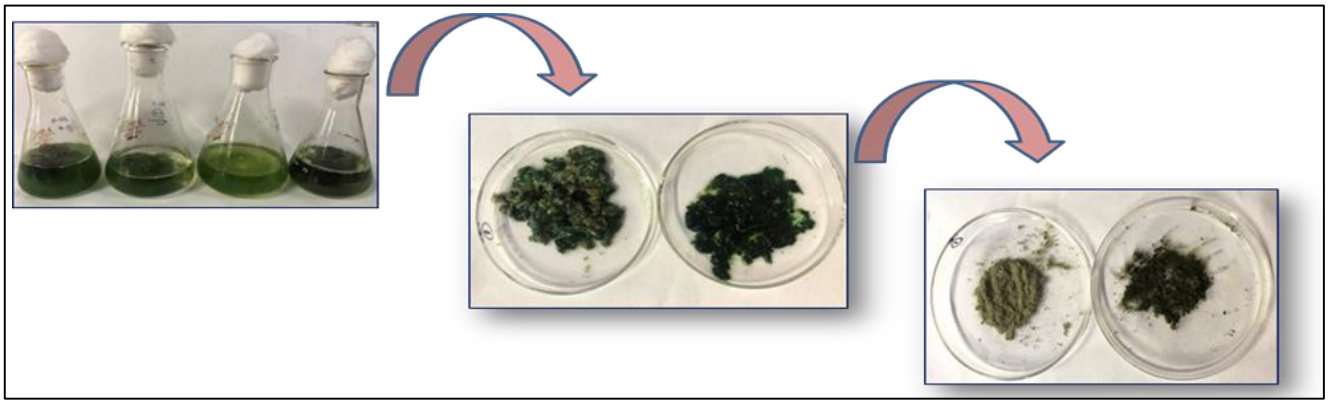

Fig 6: Microalgal biomass production

The maximum biomass was produced by isolate KMA-1 i.e. 4.85 g/L in BG-11 medium supplemented with 30% whey having dry weight of 2.60 g/L followed by HMA-1 and KMA-2 which produced 4.60 and 4.43 g/L biomass respectively (Table 3).

Table 3: Biomass production by microalgal isolates

Locale	Isolates	BG-11 Medium		BG-11 + Dairy waste (10% v/v)		BG-11 + Dairy waste (20% v/v)		BG-11 + Dairy waste (30% v/v)	
		Wet Weight (g/L)	Dry Weight (g/L)	Wet Weight (g/L)	Dry Weight (g/L)	Wet Weight (g/L)	Dry Weight (g/L)	Wet Weight (g/L)	Dry Weight (g/L)
Kurukshetra	KMA 1	3.93	1.98	4.24	2.32	4.78	2.56	4.85	2.60
	KMA 2	3.89	1.24	4.13	1.39	4.32	1.51	4.43	1.57
	KMA 3	3.28	0.90	3.69	1.09	3.97	1.20	4.07	1.24
Pehowa	PMA 1	3.57	0.97	3.81	1.10	4.09	1.31	4.13	1.33
Narwana	NMA 1	2.74	0.86	3.02	1.00	3.35	1.18	3.41	1.20
Hisar	HMA 1	3.86	1.57	4.11	1.71	4.52	2.03	4.60	2.06

All the microalgal isolates were tested for lipid content using Nile Red staining technique. The samples were taken from the cultures that have been grown for 21 days. The use of Nile Red for neutral lipid content has gained much attention over the last decade. Determination of lipid was done using Fluorescent microscopy. The algal cells when infused with Nile Red showed yellow fluorescence. Out of six isolates, five isolates showed fluorescence which were categorized in three groups: High, moderate and low lipid producers. Microalgal strains have been examined for lipid production by various researchers. High fluorescence was shown by KMA-1 as compared to other isolates and it indicated the presence of lipids. Isolate PMA-1 showed no fluorescence (Table 4, Fig 7).

Table 4: Fluorescence in different microalgal isolates

Isolate	Fluorescence
KMA 1	+++
KMA 2	++
KMA 3	+
PMA 1	-
NMA 1	+
HMA 1	++

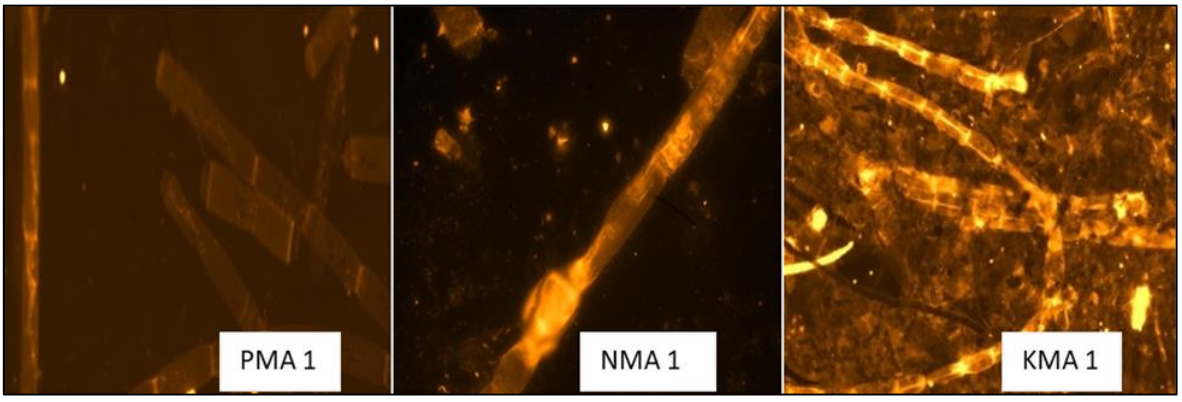

Fig 7: Microalgal isolates showing yellow fluorescence under fluorescent microscope using Nile red staining

Conclusion

All the six microalgal isolates grew better and produced higher biomass when the medium for their growth was supplemented with dairy waste. Out of six isolates, two isolates *i.e.* KMA-1 followed by HMA-1 produced 4.85 g/L and 4.60 g/L biomass, respectively, using 30% (v/v) dairy waste along with BG-11 medium. Nile red staining and fluorescent microscopy revealed highest lipid content in KMA-1 isolate whereas PMA-1 isolate was least productive. Thus, the microalgal isolates showing good growth as well as lipids can further be used for biodiesel production via trans-esterification.

Acknowledgements

The authors gratefully acknowledge the Department of Microbiology, CCS Haryana Agricultural University, and Hisar, India for providing all sort of facilities to carry out the research work.

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