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Original Article

SCREENING AND GROWTH KINETIC STUDIES OF WILD CHLOROPHYCEAN FRESH WATER MICROALGAL SPECIES FOR BIOMASS AND BIOFUEL APPLICATIONS

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ABSTRACT

Objective: Microalgae are studied for decades for various products such as, protein rich animal/fish feed, lipids, pigments, neutra ceuticals, therapeutic agents, primary products and biomass. Lipid content was prime target in most of the research programs for production of biodiesel as an alternate to fossil fuel. Chlorophycean microalgae has the potential to meet all these requirements. The objective of this study was to collect and identify chlorophycean microalgae from various water bodies of Jharkhand State of India and to estimate their total lipid content.

Methods: Wild cholorophycean fresh water species from Jharkhand were collected and studied for biomass, total lipid, carotenoids and chlorophyll content. Fourier transform infrared spectroscopy (FTIR) data were obtained for further verification of lipid estimation in all the species. Light microscopy as well as Scanning Electron Microscopy (SEM) was performed to identify the species.

Results: The observation revealed two groups of micro algae, among these *Scenedesmus* sp and *Chlorella* sp. Showed highest lipid accumulation of 45.1 and 41.5 % respectively, while *Legerhemia* sp. Showed highest biomass production (21.2 g/l). Productivity/day for an 80K L pond system was calculated by extrapolation of results; that changed the choice of organism to *Desmodesmus* sp.

Conclusion: The microalgae collected from highly polluted sites were efficient enough to yield high lipid (AKS-1/AKS-8) and biomass (AKS-6). The laboratory scale study was extrapolated with mass scale culture data and the choice of organism changed to AKS-16 from AKS-1/AKS-8 (for high lipid content) or AKS-6 (for high biomass).

Keywords: Biofuel, Chlorophyceae, Lipid, Microalgae, Productivity.

INTRODUCTION

Microalgae are getting more and more attention in various research programs globally. These include studies on microalgae as fish feed and protein rich animal diet, as a source of various nuetraceuticals, as a source of alternative energy by lipid production, as well as for wastewater treatment, hydrogen production, nano-particle synthesis, besides many more [1]. One of the most sought after research aspect is the capability of high quantities of lipid production by micro algae that can subsequently be used for biofuel production in optimized processes. Soaring prices of crude oil, fast depleting coal like non-oil energy resources globally, and increasing demand of energy per capita are the main driving forces towards the search of alternative sources of energy. Out of all alternative sources of energy; i.e. solar, tidal, hydro, thermal, geo-thermal, nuclear, wind, and biological; the biological sources are the most promising, economically feasible, long lasting and environmental friendly [2]. Among the biological 'producers' of energy, micro algae score better because of its high adaptability, high growth rate, relatively high lipid content, no or very less production of secondary metabolites, simple overall composition, abundance of species, simpler growth conditions, least dependence on various nutrient supplies, moderate resistance to *p*H change, non pathogenic, carbon neutral and environment friendly, easy to harvest and simple on post harvest processing. Indirect advantages of use of micro algae in alternative energy production will help reducing use of crop plants in such programs globally i.e. ensuring more food security, and will not use arable land for energy production; unlike energy yielding non-crop plants. Lipid production and its subsequent conversion to biodiesel ideally require a species that should have moderate to high lipid content on dry weight basis, high growth rate, and should have only moderate requirements of balanced nutrients.

Many species notably *Botryococus braunii*, *Scendesmus* sp., *Nanochloropsis* sp., *Chlorella* sp., *Phaeodactylum* sp. etc., was used in different research programs on biofuels. The biological aspect of biofuel research requires more number of species to be included in ongoing research programs. The current study is focused on searching for new species from wild and unexplored environments, and from the environments having industrial effluents. The later aspect is thought to provide comparatively more adapted pollutant resistant species which can subsequently be used for integrated research programs on wastewater treatment and lipid production simultaneously.

Fig. 1: Self made Sample Collection Device

MATERIALS AND METHODS

Sample collection

Methodology

Samples were **c**ollected from 2009 to 2012 in the months of September to December, and January to April each year repeatedly. Approximately 226Km^2 area between 22° 47' 28.30" N - 23° 40' 35.90" N and 84° 16' 5.98" E - 86° 15' 37.07"E was covered for the sample collection which included sampling from 5 dams, 4 waterfalls, 4 lakes, 3 rivers, and few other smaller water bodies (Table 1). Screw cap 100 ml bottles (Schott Duran amber/plain transparent), were used for collection with the help of a self made sample collection device. It consisted of four PVC pipes each of 54

cm length with a thread connector at both the ends and the head piece having a burette holder clamp at distal end. Using this device samples were collected from different sites and at different depths up toone meter. With its full length it facilitates collection away from the shores, once dismantled can be easily kept in a small shoulder bag and is very light weight, durable and non corrosive (fig. 1).

Table 1: Sampling details

Area visited	Coordinates	First collection date	Light microscopic identification	Successfully isolated species
Swarnrekha river	23.407931 N, 85.440731 E	13/06/09	*Closterium*	
			Cosmerium	
			Scenedesmus	*AKS-20*
			Franceia	
Jumar river	23.411378 N, 85.402136 E	17/07/09	*Gonyostomum*	
			Monoraphidium	AKS-5
Hundru Fall	23.450869 N, 85.667170 E	12/10/10	*Chlorococcum*	
			Desmodesmus	AKS-18
Dimna Lake	22.860638 N, 86.260299 E	07/05/10	*Desmodesmus*	AKS-7
			Scenedesmus	AKS-1
			Botryococcus	AKS-12
			Desmodesmus	AKS-13
Geetalsud Dam	23.458385 N, 85.527749 E	12/10/10	*Scenedesmus*	AKS-19
Rukka Dam	23.441834 N, 85.480065 E	07/04/11	*Ankistrodesmus*	
			Chlamydomonas	
			Bracteococcus	AKS-3
Jonah Falls	23.341667 N, 85.608333 E	21/03/09	*Chlorella*	AKS-8
			Kirchneriella	
Jublee park	22.812402 N, 86.197325 E	05/02/10	*Tetrallantus*	
Patratu Dam	23.615557 N, 85.291189 E	05/03/09	*Selenestrum*	
			Scenedesmus	AKS-17
Ranchi Lake	23.368364 N, 85.318145 E		*Lagerheimia sp.*	AKS-6
			Ankistrodesmus	
Hirni Falls	22.866700 N, 85.3333 E	09/01/10	*Ecballosystopsis*	AKS-4
			Lagerheimnia	
Panchghagh Falls	22.952900 N, 85.267907 E	09/01/10	*Desmodesmus*	AKS-11
			Closteridium	
Tata Steel Lake	22.791197 N, 86.191703 E	12/09/09	*Scenedesmus*	AKS-2
			Chlamydomonas	AKS-10
Bokaro Steel cooling pond 2	23.676640 N, 86.13701 E	05/04/10	*Maesotaenium*	
Damodar River, Ramgarh	23.641015 N, 85.510443 E	05/04/11	*Gymnodinium*	
			Scenedesmus	
			Desmodesmus	AKS-14
Netarhat	23.479012 N, 84.268329 E	05/06/11	*Desmodesmus*	AKS-15
Badka Bandh			*Desmodesmus*	AKS-16
			Desmodesmus	AKS-9

Sampling, and determination of mineral composition of water samples

Fresh bottles were used for collection; bottles were labeled with date, time, depth, and site of collection. For proper gaseous exchange bottles were filled up to two third volumes only. Sample waters were analyzed for mineral composition using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer), Perkin Elmer, USA; Optical 2100DV equipped with UV sensitive dual back side – illuminated CCD Array detector. This was specifically done to select the 'type culture medium' and development of minimal media composition for wild culture maintenance subsequently. Additionally, soil extract media was also prepared to supplement the wild culture as and when required as per culture media composition provided on University of Texas (UTEX) website (Table 2) [3]. The composition of same was also analyzed using ICP-OES. Approximate 0.3 gm of samples were digested by using 5 ml HNO_3(65% Suprapur, Merck) and 1 ml H_2O_2(30% Suprapur, Merck) in a multi wave 3000 microwave digestion system (Anton Paar, Austria).

Samples storage and conditions

Collected samples were first stored at Green house under standard conditions (25 ^{0}C; Relative Humidity: 80;Photoperiod: 16h/8);before bringing them to the Algal culture facility (ACF). Wild samples were screened and using standard isolation techniques. Axenic cultures were developed and were maintained in the ACF. Light microscopic studies and morphological identification was done using published article and the available algal monographs [4].

Sample maintenance and media supplementation

Collected wild samples were maintained in sample site waters and soil extract media till isolation of species. Isolated species were then transferred to modified Bold's basal 3N media (Table 2) [4]. All the media constituents were of analytical grades. For soil extract media, soil was collected from the Institute campus where human interference and agriculture activities are null. Collected soil was crushed using pestle and mortar, and heated twice at 160°C for 3 hr to eliminate the entire living microorganism as well as spores (as suggested in UTEX website). After cooling, 50 g of soil was transferred to 300 ml distilled water and autoclaved twice at an interval of 24h at 150°C for 35 minutes to eliminate fungal spores. Supplemented samples were maintained in the ACF at 25±2°C under 6300 lux light intensity with 16:8 photoperiod.

Isolation

Depending upon the motility and other attributes of the collected samples following isolation techniques were used for obtaining isolates (fig. 2A).

Table 2: Media Composition (Modified Bold's Basal 3N Media)

#	Component	Final Concentration
1	$NaNO_3$	8.82 mM
2	$CaCl_2 \cdot 2H_2O$	0.17 mM
3	$MgSO_4 \cdot 7H_2O$	0.3 mM
4	K_2HPO_4	0.43 mM
5	KH_2PO_4	1.29 mM
6	NaCl	0.43 mM
7	Soil water: GR+ Medium	40 mL/L
8	Vitamin B_{12}	0.135 mg/L
9	Biotin Vitamin Solution	0.025 mg/L
10	Thiamine Vitamin Solution	1.1 mg/L
11	P-IV Metal Solution	
a.	$Na_2EDTA \cdot 2H_2O$ (Sigma ED255)	2 mM
b.	$FeCl_3 \cdot 6H_2O$	0.36 mM
c.	$MnCl_2 \cdot 4H_2O$	0.21 mM
d.	$ZnCl_2$	0.037 mM
e.	$CoCl_2 \cdot 6H_2O$	0.0084 mM
f.	$Na_2MoO_4 \cdot 2H_2O$	0.017 mM

Serial dilution [5]

Serial dilution method was used for isolation of algal species up to 10^{-}[10] dilution from collected samples aseptically and pure species were maintained in modified Bold's Basal 3N media.

96 Well culture plate techniques [6]

The enriched culture was diluted suitably using media and 200 µl of diluted samples were transferred to each 300 µl capacity well of 96 well plates and incubate at 25±2°C under 6300 lux illumination for isolation of different algal species(fig. 2B).

Streak plating [5]

Petri dishes containing solidified Bold basal 3N growth medium were streaked from mixed cultures to isolated non motile species. A drop of the mixed culture was placed near the periphery of the plate. Using aseptic techniques sterile loop was used to make streaks of the suspension on agar plate. Petri dishes were covered and sealed with parafilm and incubated under 6300 lux light intensity at 25±2°C with 16:8 hrs photoperiods. Individual colonies were isolated and purified by several streaking, and inoculated into liquid medium again and re-incubated at 25±2°C under 6300 lux light intensity with 16:8 hrs photoperiod(fig. 2C).

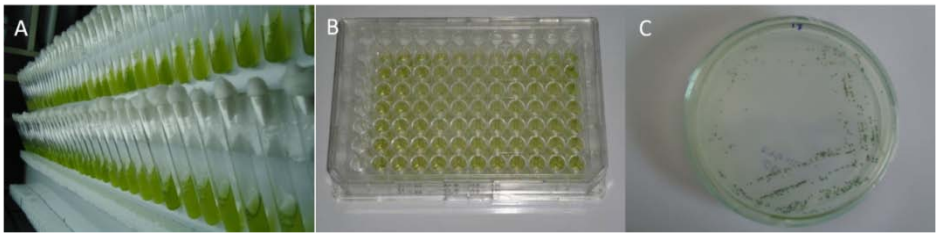


Fig. 2: Techniques used to develop pure culture

Antibiotic treatment

Monocultures of alga thus obtained through any of the above mentioned isolation techniques were subsequently given the antibiotic and anti fungal treatments (200 mg/l Penicillin, 100 mg/lS treptomycin, 20 mg/l Chloramphenicol and 3.5 mg/l Nystatin)[7,8].

Morphological identification of species

Identification of different species was carried out using light (Olympus CH20, Japan) as well as scanning electron microscopy(SEM; JEOL JSM 6390LV, Japan). For this, 1 ml of 15 days old samples were taken in 2 ml eppendorf tubes and centrifuged(Eppendorf 5804R, Germany) for 5 min at 5000 rpm. Pellets obtained were washed twice with distilled water followed by addition of 1 ml of 2.5% glutaraldehyde and the samples were incubated for one hour at room temperature. Glutaraldehyde was removed by using centrifugation at 3000 rpm. Dehydration was carried outwith ethanol series (25%, 50%, 75%, 95% and 100%). Dehydrated algal samples were coated with gold in sputter system(JEOL JFC 1600, Japan) for 30 minutesand then used for SEM study.[9]

Batch cultivation of algal species

All the 20 isolated algal species were grown axenically for further experimentation in 250 ml flasks containing their respective medium with 10% (v/v) inoculums. Flask containing medium was sterilized in an autoclave for 15 min at 121°C to avoid any contamination. Cultures were maintained in ACF equipped with temperature, light intensity, and photoperiod control. Photosynthetic Active Radiation (PAR) tubes were used for continuous illumination at 6300 lux, at 16:8 hrs on- off cycle. Cultures were maintained at 25±2°C, and were shaken manually 2-3 times a day.

Estimation of biomass, total lipids, and pigments

For the estimation of growth of all algal species absorbance at 680 nm was taken at a 5 days interval using spectrophotometer (PerkinElmer Lamda 45, Germany), till stationary phases reached by all cultures. Cells were harvested at stationary phases by centrifugation at 8000 rpm for 10 min. The supernatant was discarded and pellet was washed twice with distilled water. Respective pellets were dried at 50°C till constant weight was obtained, and dry weights were recorded.

Total lipids from the pellets were extracted by using a mixture of chloroform, methanol, and water, as per Bligh and Dyer method [10].

For chlorophyll estimation, 1 ml of algal broth of each culture was taken in 2 ml of centrifuge tube and centrifuged at 5000 rpm for 5 min. Supernatant was discarded and 1 ml of acetone was added, and incubated overnight. The extract was centrifuged at 3000rpm for 2 min. and chlorophyll *a*,chlorophyll *b* and total chlorophyll content

was measured by taking absorbance at 662 nm and 645 nm respectively using the following equations, while total carotenoid was estimated by taking absorbance at 470 nm.[11]

$$a = (12.7 \times A_{662}) - (2.69 \times A_{645}) \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (i)$$
$$b = (22.9 \times A_{645}) - (4.64 \times A_{662}) \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (ii)$$
$$a + b = (8.02 \times A_{662}) - (4.64 \times A_{645}) \cdots\cdots\cdots\cdots\cdots\cdots (iii)$$
$$c = (1000 \times A_{470} - 2.270 \times a - 81.4 \times b) \div 227 \cdots\cdots (iv)$$

Where A= absorbance, a= chlorophyll a, b= chlorophyll b, a+b= total chlorophyll and c= total carotenoid

Fourier Transform Infrared Spectroscopy (FTIR) Studies

Comparative study of total lipids present in all the isolates was reassessed through FTIR spectroscopy. FTIR is used to analyse the structure and chemical bonding of a compound, particularly functional groups. One milligram of dried algal biomass for each isolate was blended with 99 mg of potassium bromide(KBr) and pressed into tablets before measurement. FTIR attenuated total reflectance was collected on Shimatzu IR-prestige 21 instrument with mercury cadmium telluride (MCT) detector. A region of 4000 cm^{-1} to 400 $cm^{-1}$was recorded for scanning of functional groups.

The peaks at the region of 2928 cm^{-1}, 2860 cm^{-1} and 1740 cm^{-1}that are used to estimate total lipid content, were carefully fitted and calculated.[12,13] Data was analysed using Origin pro-8 software.

RESULTS AND DISCUSSION

ICP-OES data analysis

All the water samples were found to have near similar composition to water from Swarnrekha River; which is one of the collection sites, the mineral composition is presented here in Table 3. The analysis of soil extracts media using ICP-OCP also presented in Table 4.

Table 3: ICP results showing Swarnrekha River water analysis

Analyte		Mean Corrected Intensity	Calibration		Sample		
			Concentration	Stv Dev	Concentration	Stv. Dev.	RSD
Ba	233.52	74671.8	0.064	0.007	0.064	0.0007	1.03
Mg	285.21	22355153.7	12.02	0.015	12.02	0.013	0.13
Li	670.784	629205.7	0.016	0.0001	0.018	0.0001	0.3
Mn	257.61	51607.1	0.01	0.0004	0.01	0.0004	4.25
Ni	231.6	-15.1	0	0.0003	0	0.0003	131.46
Zn	206.2	23890.6	0.116	0.0007	0.116	0.0007	0.56
As	193.69	27.3	0.005	0.0047	0.005	0.0047	95.38
K	766.49	35749970.7	18.97	0.046	28.97	0.048	0.17

Table 4: ICP results showing soil water analysis

Analyte		Mean Corrected Intensity	Calibration		Sample		
			Concentration	Stv. Dev.	Concentration	Stv. Dev.	RSD
Ba	233.52	105779.9	0.092	0.0002	0.092	0.0002	0.24
Mg	285.21	14967975	8.05	0.0339	8.05	0.0339	0.42
Li	670.784	465164.8	0.015	0.0001	0.015	0.0001	0.43
Mn	257.61	1278144	0.25	0.0009	0.25	0.0009	0.36
Ni	231.6	-391.4	-0.002	0.001	-0.002	0.001	62.94
Zn	206.2	29725.3	0.152	0.0011	0.152	0.0011	0.7
As	193.69	17.1	0.003	0.0053	0.003	0.0053	166.79
K	766.49	15156962.6	12.2	0.189	12.28	0.189	1.54

Identification of isolated species

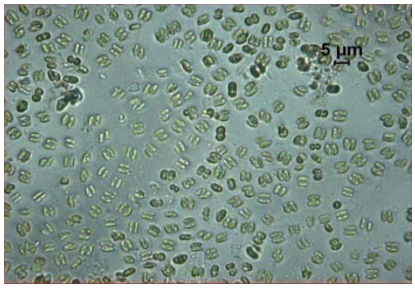
In the primary screening a total of 21 genera namely; *Ankistrodesmus, Botryococcus, Bracteococcus, Chlamydomonas, Chlorella, Chlorococcum, Closteridium, Closterium, Cosmerium, Desmodesmus, Ecballosystopsis, Franceia, Gonyostomum, Gymnodinium, Kirchneriella, Lagerheimia, Maesotaenium, Monoraphidium, Scenedesmus, Selenestrum, and Tetrallantus;* of Chlrophycean micro algae were found predominantly in most of the water bodies of this unexplored area of Chotanagpurdry deciduous ecoregion. Out of these 21 genera, 20 species for seven genera were isolated successfully. Light microscopy and SEM of these isolates are presented in fig. 4 and 5 respectively. All the species were maintained at optimal conditions in ACF.

Growth profiling of isolated species

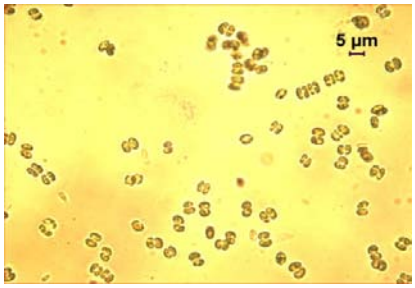
Growth profiling of all the species in Bold's basal 3N media showed that as the incubation time increases the cell growth (Absorbance) also increased and reached to a maximum concentration at stationary phase which is in between 45^{th} to 55^{th} days (fig. 6).

The total cell biomass (dry cell weight) and total lipids of all the isolates were recorded at stationary phase presented in table 5. At stationary phase the isolate AKS-6 showed the highest biomass (21.2 g/l) while AKS-13 showed least biomass accumulation (0.8 g/l) on dry weight basis. The highest lipid accumulation was observed in AKS-1 (45.1%) followed by AKS-8 (41.47%) and AKS-16 (28.44%). This shows that AKS-1 and AKS-8 are the two most promising candidates for lipid production programs. AKS-1 is a Scenedesmus species while AKS-8 is a Chlorella Species. The lipids produced by these two species are in the range of high lipid producing species globally [14], and best reported in autotrophic cultures so far, in contrast to heterotrophic cultures as studied by Ren et al (2013) [15]. This makes these species the best reported candidate for lipid production at minimal media without any supplementary carbon source.

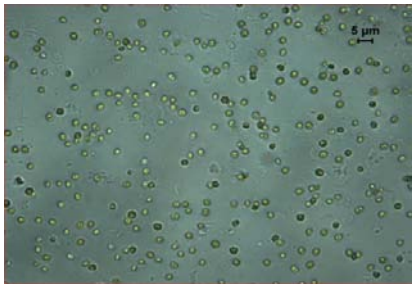
The growth profile of selected species also showed that total chlorophyll and presence of carotenoids reached to their maximum concentration at stationary phase as showed in fig. 7 and8 respectively. The observation of growth, total chlorophyll and total carotenoids by all the isolates showed two clear patterns that has been demarcated as group A (AKS-2, AKS-4, AKS-5, AKS-7, KAS-8, AKS-10, AKS-15 and AKS-20) and Group B (AKS-1, AKS-3, AKS-6, AKS-9, AKS-11, AKS-12, AKS-13, AKS-14, AKS-16, AKS-17, AKS18, and AKS-19). The growth profiling of all the isolates belonging to show that Group A was is a group of fast grower isolates while Group B members were slow growers initially. The latter group showed slow growth till 40^{th} day and then a steep rise in cell biomass, total chlorophyll, and total lipid accumulation, where AKS-4 was found to be the fastest grower while AKS-6 was the slowest grower. This sudden rise in all group B members may be accounted for some complex biochemical mechanisms occurring in them. The results shown here further indicate a fact that the slow growing species provide good results in terms of total lipid produced if cultured for prolonged time, so the total yield per unit over time will be higher than the fast growers.



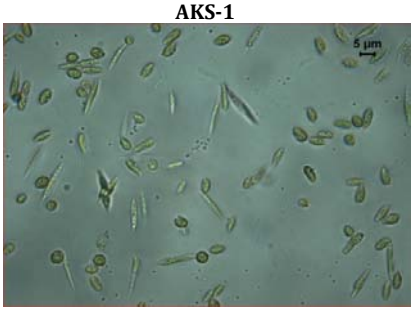
AKS-1



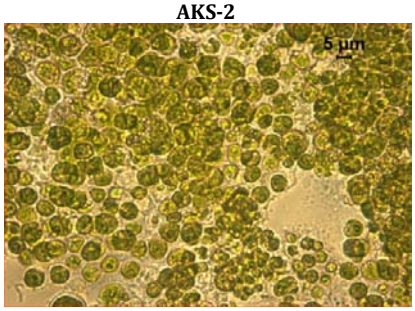
AKS-2



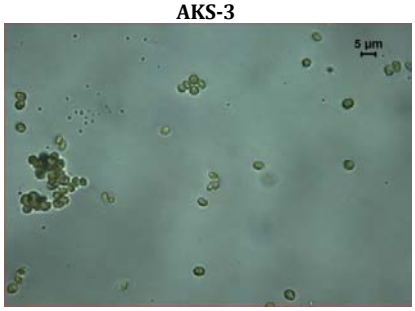
AKS-3



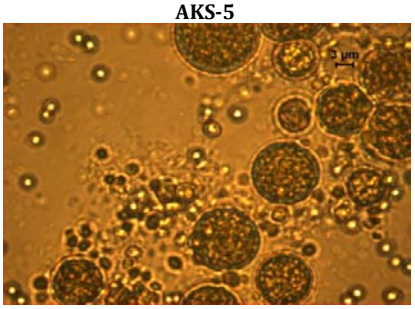
AKS-5



AKS-6



AKS-7



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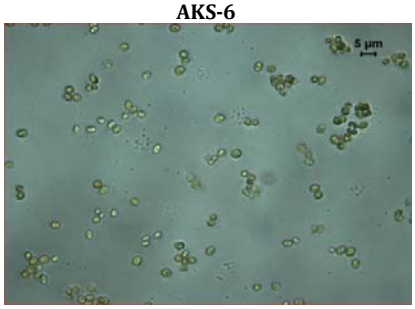

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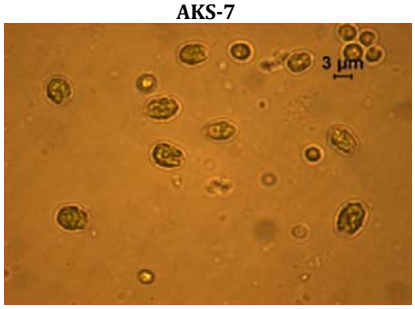

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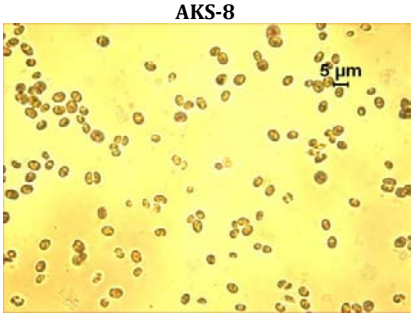

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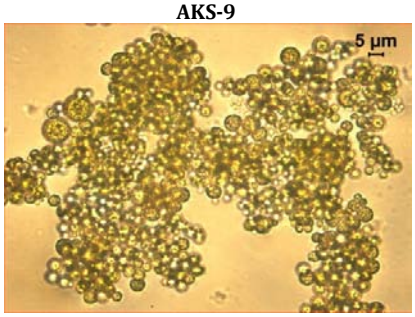

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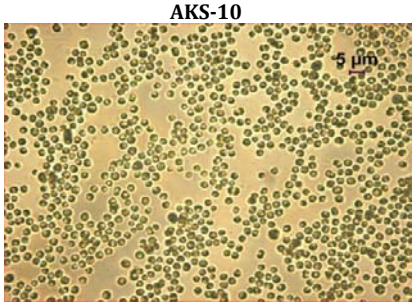
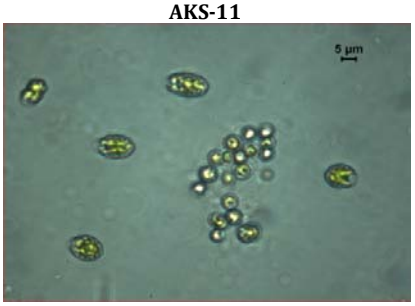
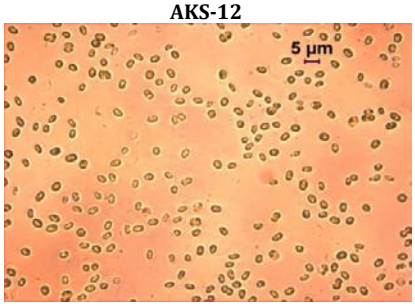


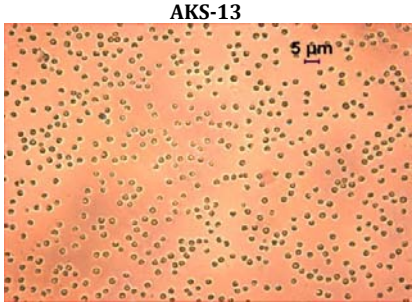
AKS-13



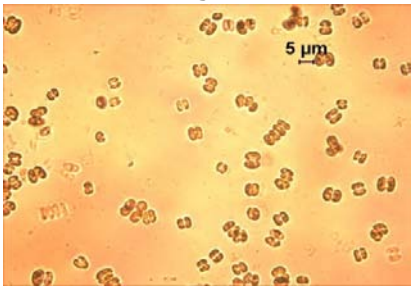
AKS-14



AKS-15



AKS-16



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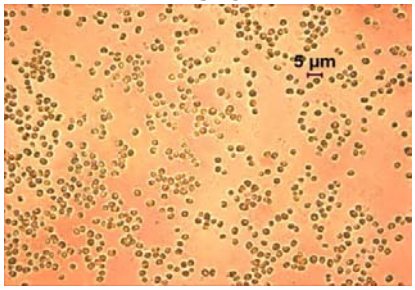

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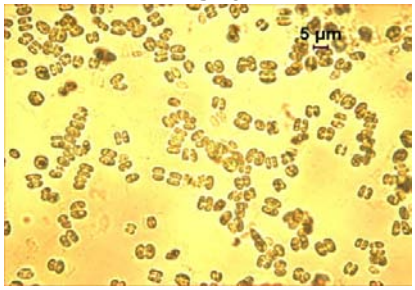
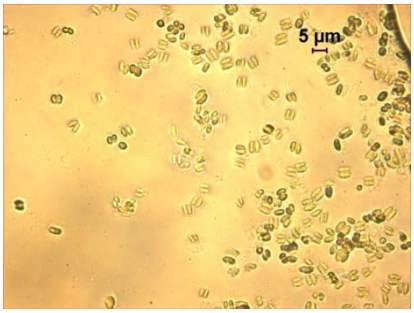
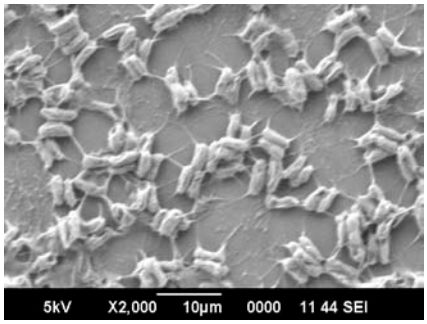


AKS-19

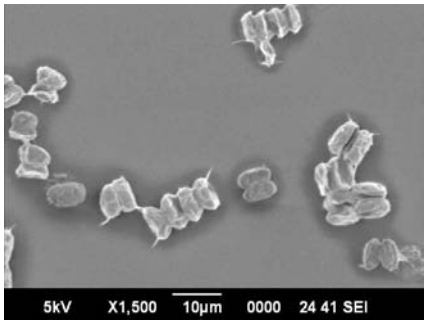


AKS-20

Fig. 4: Light microscopy of isolated species



AKS-1



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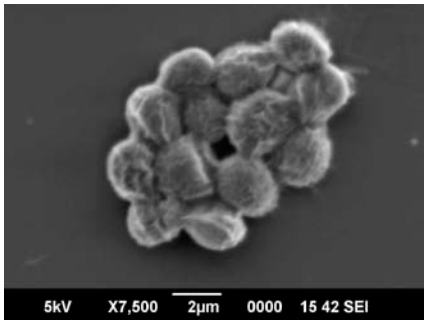
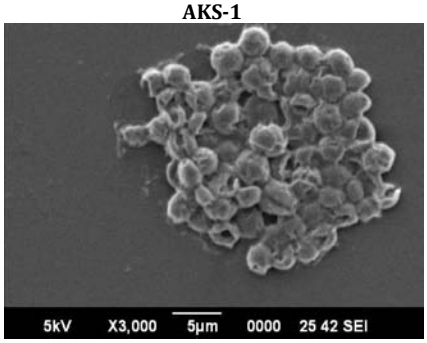


AKS-3



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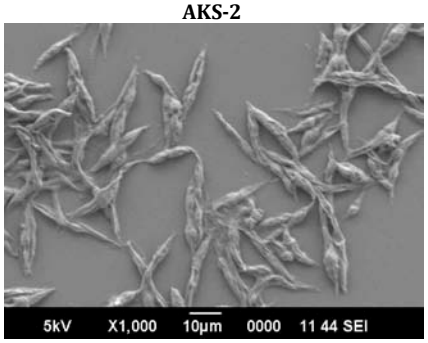

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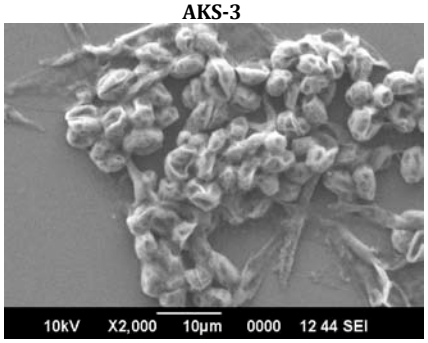

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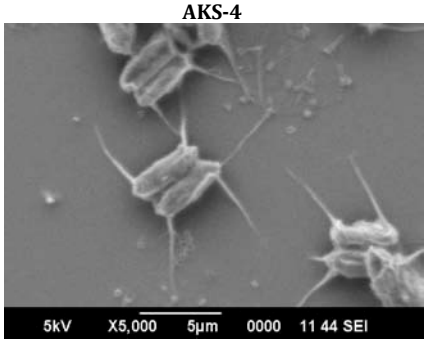

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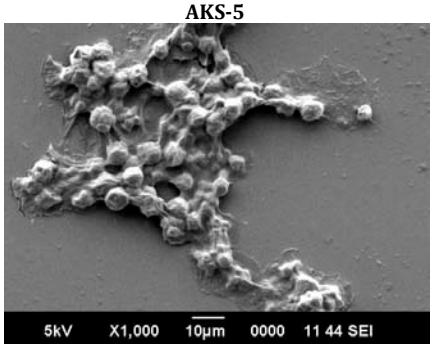

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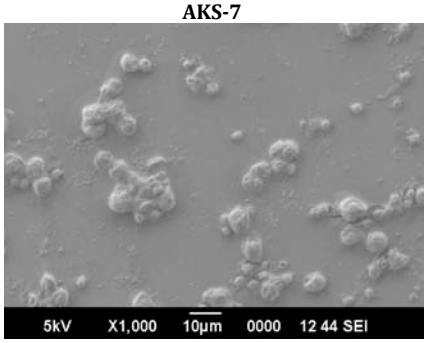

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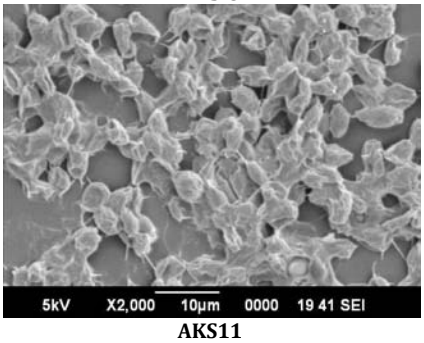
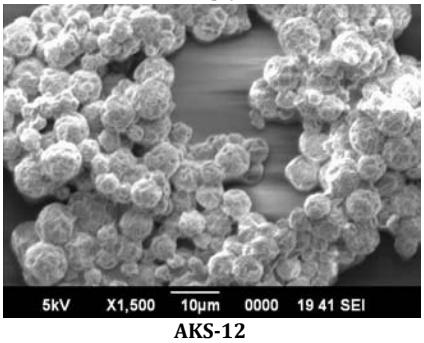
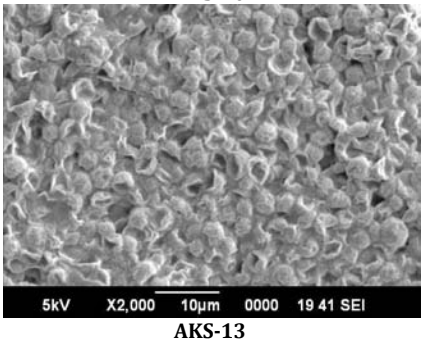


AKS11



AKS-12



AKS-13

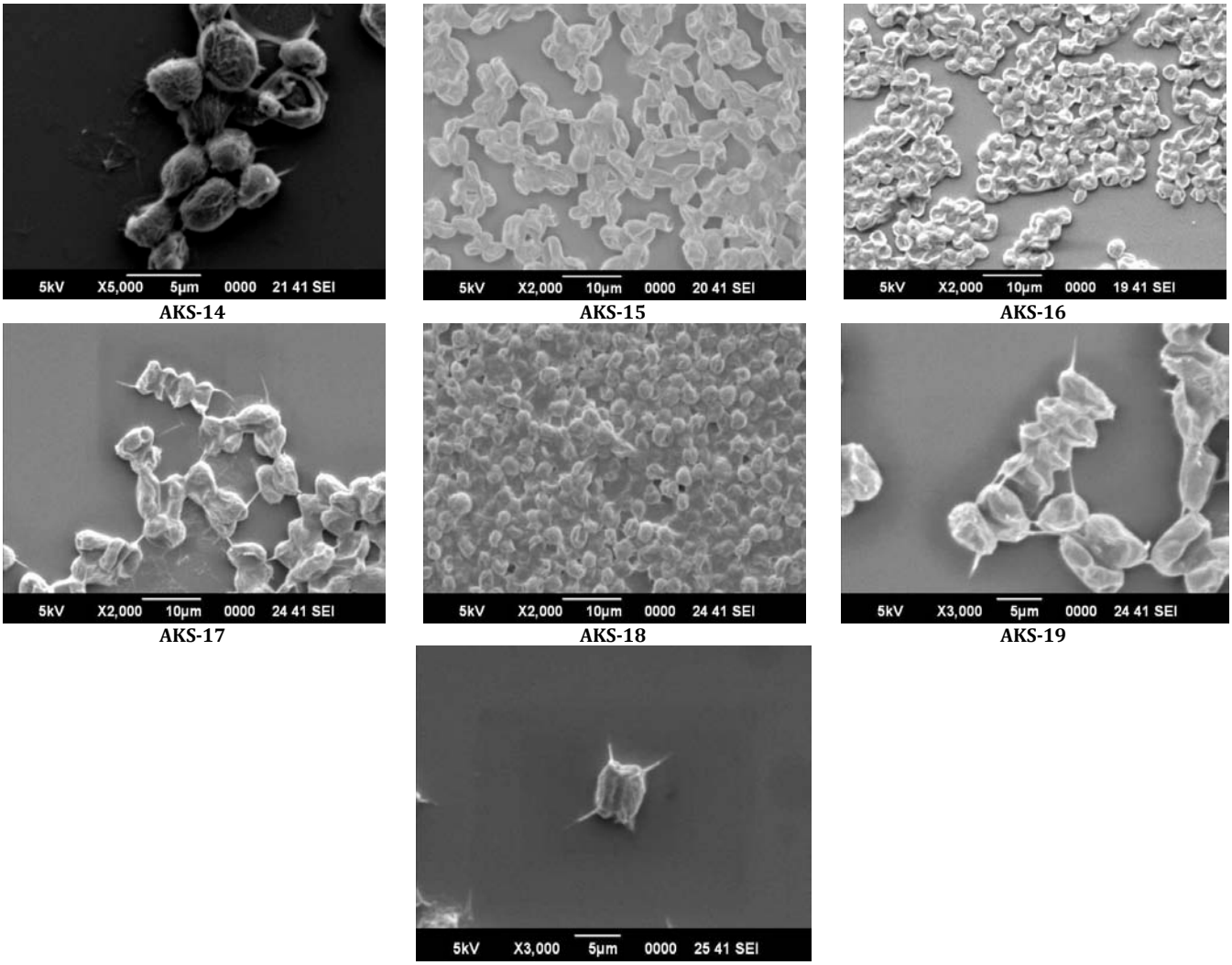


Fig. 5: Scanning electron microscopy of isolated species

Table 5: Details of Yields of pigments over biomass, total biomass, and lipid content of the isolates; and extrapolations to 80K Liter pond system

Group	Isolate	Yield of Chlorophyll over Biomass (mg/g)	Yield of Carotenoid over Biomass (mg/g)	Biomass (g)/ Liter	% of lipid on dry weight basis	weight of lipid (kg) in 80K L pond	Days to Stationary Phase (Day to Harvest)	Average Yield (Kg) in 80K pond per day
B	AKS 1	21.97	0.37	1.68	45.15	6068.16	55	110.33
A	AKS 2	66.1	1.77	0.49	2.54	99.56	55	1.81
B	AKS 3	8.7	0.1	4.64	5.85	2171.52	60	36.19
A	AKS 4	14.06	0.67	3.47	16.3	4524.88	55	82.27
A	AKS 5	2.92	0.19	9.89	6.73	5324.77	45	118.32
B	AKS 6	0.71	0.03	21.2	2.7	4587.68	45	101.94
A	AKS 7	14.22	0.48	2.94	20.52	4826.3	55	87.75
A	AKS 8	20.04	1.18	1.19	41.47	3947.94	35	112.79
B	AKS 9	3.58	0.06	9.75	3.64	2839.2	65	43.68
A	AKS 10	1.7	0.06	16.09	2.36	3037.79	50	60.75
B	AKS 11	18.99	0.33	1.84	25	3680	60	61.33
B	AKS 12	6.45	0.13	3.97	5.45	1730.92	55	31.47
B	AKS 13	50.76	0.78	0.8	25.31	1619.84	60	26.99
B	AKS 14	4.75	0.08	10.13	3.47	2812.08	60	46.86
A	AKS 15	18.62	0.81	2.45	24.08	4719.68	55	85.81
B	AKS 16	12.19	0.43	3.96	28.44	9009.79	65	138.61
B	AKS 17	27.17	0.42	1.34	23.73	2543.85	65	39.13
B	AKS 18	6.57	0.13	4.45	11.28	4015.68	60	66.92
B	AKS 19	13.76	0.27	2.27	23.28	4227.64	60	70.46
A	AKS 20	8.8	0.43	4.43	4.54	1608.97	45	35.75

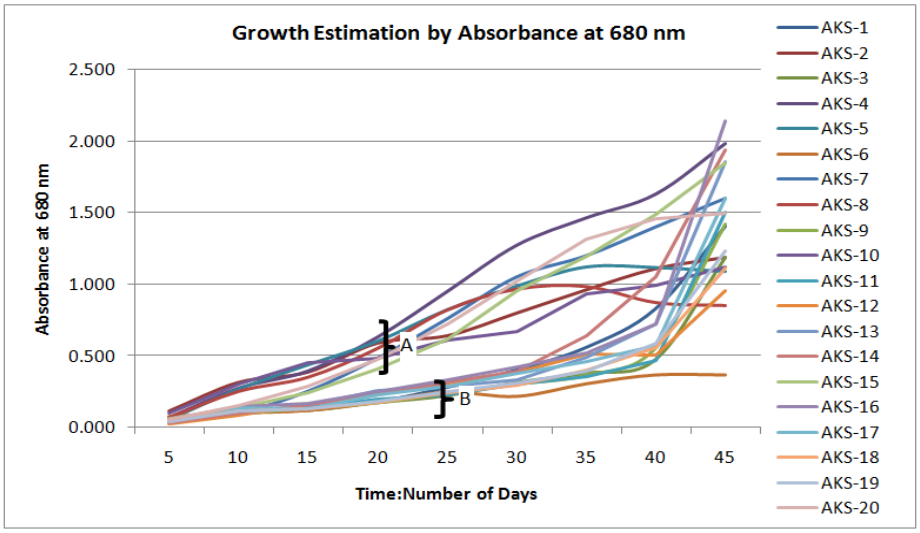


Fig. 6: Growth pattern of isolated species

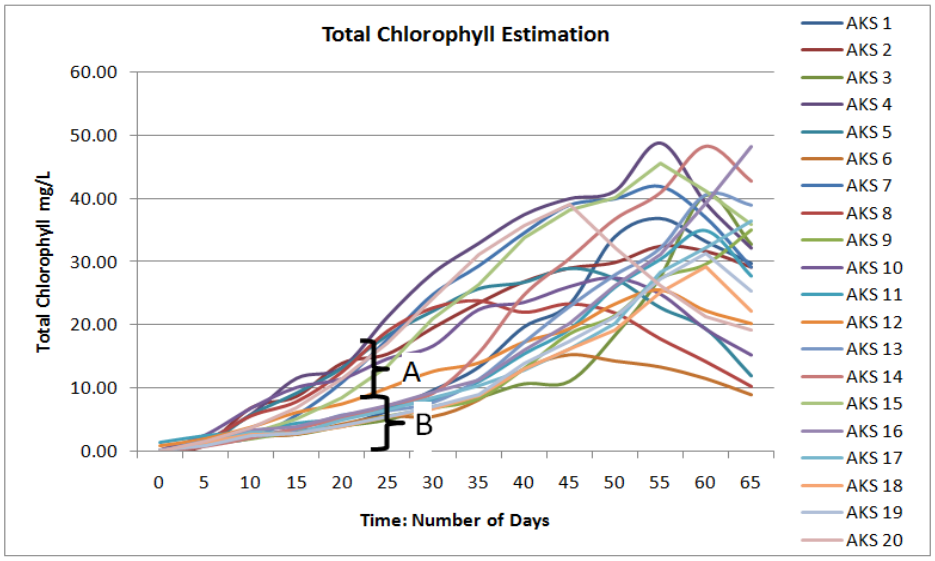


Fig. 7: Total chlorophyll content of the isolated species

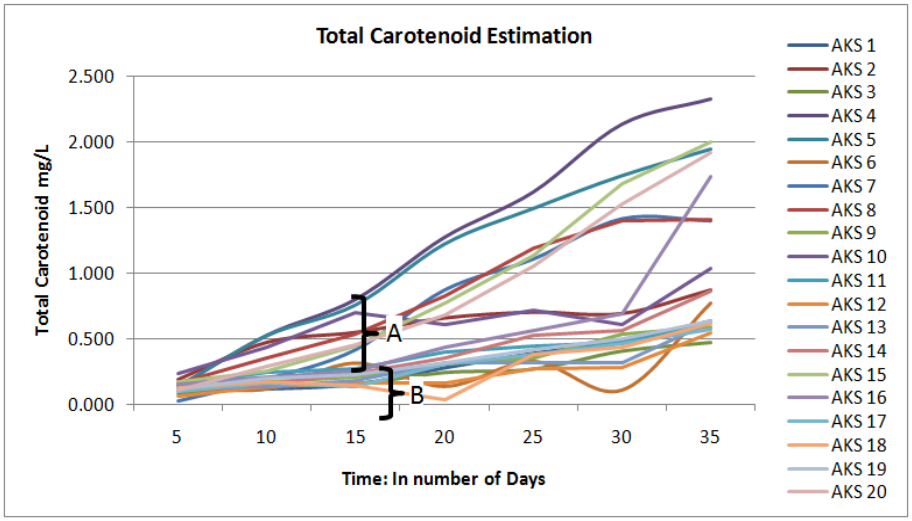


Fig. 8: Total carotenoid content of isolated species

Yields calculated for total chlorophyll, total carotenoid, and percent total lipids, over biomass are shown in Table 5. The maximum carotenoid and chlorophyll per unit of biomass was highest by AKS-2 followed by AKS-13. Yield patterns also show two clearly demarcated groups of same organisms, endorsing Group-A and Group-B.

FTIR spectrum analysis

The FTIR spectrum of algal biomass with lipid content from all the isolates is shown in fig. 9. The absorption bands in the FTIR spectra reflect the presence of hydroxyl (OH), carbonyl (C=O), alkane, alkene and amide groups in algal isolates. The region 3300- 3000 is due to O-H stretching. A CH_2 stretching vibration in the range of 3100-2800 cm^{-1} incriminated the presence of lipids[16]. A prominent band at 2924 cm^{-1} is due to asymmetric CH_2 stretching vibration, mostly due to methylene groups of lipids whereas the band at 2860 cm^{-1} assigned to symmetric CH_2 stretching vibration of methyl groups of lipids. The band at 2360 cm^{-1} corresponds to CO_2 vibration while that at 1740 $cm^{-1}$was due to C=O of ester and fatty acids. The regions 1700-1600 cm^{-1} reveals the presence of amide I bands [17], which is mainly due to C=O stretching vibration of peptide bond [18] whereas 1600-1500 cm^{-1} is specific for amide II bands, which was due to N-H bending vibration [19]. A weak band at 1240 cm^{-1} can be assigned to P=O stretching of phospho-diester backbone of nucleic acids (DNA and RNA). Declination in the intensity of absorption at 2928, 2860 and 1740 cm^{-1} reflects the decrease in lipid content.

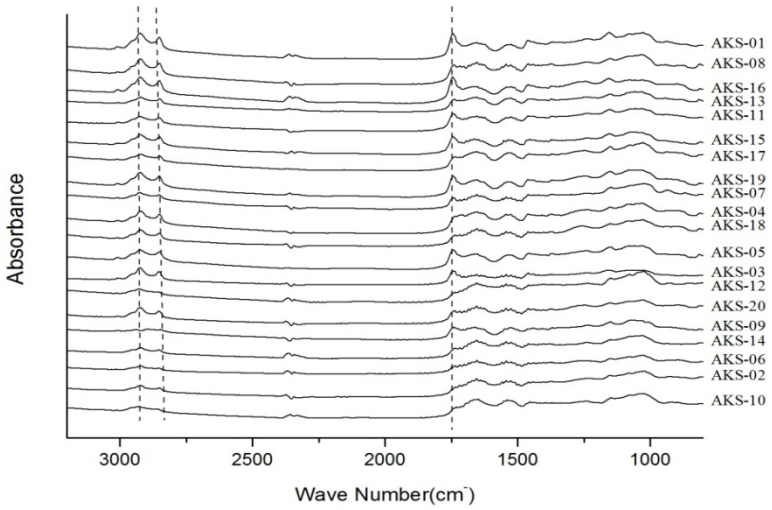


Fig. 9: Comparative analysis of the FTIR absorbance spectra of isolated species in which absorbance at 2928, 2860 and 1740 cm^{-1} shows the lipid content

Yield estimationsfor raceway pond system

For a practical application view point the higher percent of lipid production per unit biomass may not satisfy the economics, but otherwise advocated mostly. To our understanding, a moderated percent of lipid production with a higher biomass produced per liter; typically changes the scenario on the choice of the organism to be employed for any mass scale production program.

The data obtained in present investigation were extrapolated for an 80,000 L raceway pond system and the productivity per day is shown in table 5 and fig. 10. It has been observed that the organism of choice changes to AKS-16 (a *Desmodesmus*species), from AKS-1/ AKS-8 (the two highest percent lipid producers), or AKS-6 (the highest biomass producer). Although, depending on the nature and priorities of a given program, a higher biomass producer can be of choice because for the economics and potential uses of the high quantities of the leftover biomass after lipid extraction. Moreover, a higher biomass will definitely reduce the cost of nutrient recycling in any of the mass scale algae production program.

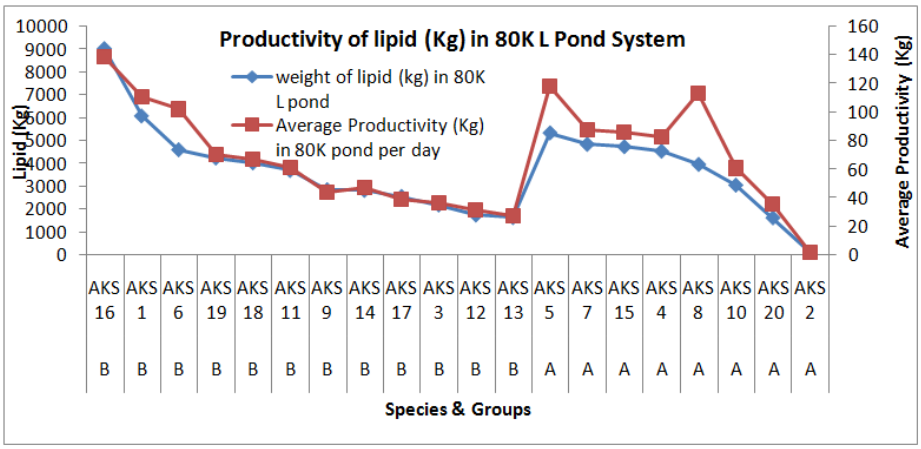


Fig. 10: Mass culturing estimates shows the slow growers outweigh fast growers

CONCLUSION

The results obtained in the present study showed that the microalgal isolates from highly polluted sites grown on minimal media in autotrophic mode are efficient enough to yield very high lipids (AKS-1/AKS-8) and biomass (AKS-6). The yield of lipid/biomass as well as pigments/biomass displayed promising results for most of the isolates. The laboratory scale studies can be extrapolated or supported with mass scale culture data for further selection of appropriate organism for scale up programs; as it has been observed in the present study, that choice of microalgae has been changed to AKS-16 (a *Desmodesmus* Species), from AKS-1/ AKS-8 (the two highest % lipid producers), or AKS-6 (the highest biomass producer). The slow growing isolates, under prolonged cultivation, showed more promising results in term of lipid content, compared to the fast growing isolates. Therefore, it would be ideal to choose slow growing isolates for long term economic benefits.

ACKNOWLEDGEMENT

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CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Chu W-L. Biotechnological applications of microalgae. Int E-J Sci Med Educ 2012;6:S24-S37.
2. Priyadarshani I, Rath B. Commercial and industrial applications of micro algae–A review. J Algal Biomass Util 2012;3(4):89-100.
3. http://web.biosci.utexas.edu/utex/mediaDetail.aspx?mediaID=147(page last visited: 17/10/2014)
4. Prescott GW. How to know: the freash water algae. WM. G BROWN company, Dubuque, IOWA http://cccryo.ntr.io/sources/files/medien/BBM.pdf(page last visited:17/02/2012).
5. Dayananda C, Sarada R, Kumar V, Ravishankar GA. Isolation and characterization of hydrocarbon producing green alga *Botryococcus brauni*ifrom Indian freshwater bodies. Electron J Biotechnol 2007;10:78-91.
6. Moris GM, Costa JAV. Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energy Convers Manage 2007;48(2):2169-73.
7. Gomez PI, Gonzalez MA. Genetic polymorphism in eight Chilean strains of the carotenogenic microalga *Dunaliella salina*. Biol Res 2001;34(1):23-30.
8. ChoiJ-S, ChoJ-Y, JinL-G, JinH-J, HongY-K. Procedures for the axenic isolation of conchocelis and monospores from the red seaweed *Porphyra yezoensis*. J Appl Phycol 2002;14:115–21.
9. Callowa JA, Osborne MP, Callowa ME, Baker F, Donald AM. Use of environmental scanning electron microscopy to image the spore adhesive of the marine alga *Enteromorpha* in its natural hydrated state. Colloides Surf 2003;27:315-21.
10. Bligh EG, Dyer WJ. A rapid method for total lipid extraction and purification. Can J Biochem Physiol 1959;37:911-7.
11. Litchtenthaller HK, Wellburn AR. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. Biol Soc Trans 1985;11:591-2.
12. Phukan MM, Chutia RS, Konwar BK, Katki R. Microalgae *Chlorella* as a potential bioenergy feedstock. Appl Energy 2011;88:3307-12.
13. Mahapatra DM, Ramachandra TV. Algal biofuel: bountiful lipid from *Chlorococcum*sp. proliferating in municipal wastewater. Curr Sci 2013;105(1):47-55.
14. Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25:294–306.
15. Ren H-Y, Liu B-F, Lei CM, Zhao, Ren N-Q. A new lipid-rich microalga*Scenedesmus* sp. strain R-16 isolated using Nile red staining: effects of carbon and nitrogen sources and initial pH on the biomass and lipid production. Biotechnol Biofuels 2013;6:143-52.
16. Stuart B. Infrared spectroscopy: fundamentals and applications. 1st ed. West Sussex: John Wiley and Sons, Ltd; 2004.
17. Dumas P, Miller L. The use of synchrotron infrared microspectroscopy in biological and biomedical investigations. Vib Spetrosc 2003;32:3–21.
18. Backmann J, Schultz C, Fabian H, Hahn U, Saenger W, Naumann D. Thermally induced hydrogen exchange processes in small proteins as seen by FTIR spectroscopy. Proteins Struct Funct Bioinf 1996;24:379–87.
19. Fischer G, Braun S, Thissen R, Dott W. FT-IR spectroscopy as a tool for rapid identification and intra-species characterization of airborne filamentous fungi. J Microbiol Meth 2006;64:63–77.