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OSSEIN EFFLUENT AS WATER AND NUTRIENTS FOOTPRINT FOR BIODIESEL FEEDSTOCK PRODUCTION IN *CHLORELLA VULGARIS* BDU G91771

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ABSTRACT

India's national policy on biofuels has proposed a non-mandatory target of 20% blend of biodiesel and ethanol to meet the country's energy demand. The microalgae is one of the most promising feedstock of biodiesel. The research has identified an Indian marine isolate *Chlorella sp.* that can produce about 17% lipid and can be grown in ossein effluent containing low total dissolved solid and to use it as microalgal growth medium, reducing the water and nutrient footprint. This gives us lead for largescale microalgal technology, where *Chlorella* sp. can be grown in a sustained manner. The effluent composition almost mimics the essential algal nutrients (growth medium) and alternatives to the nutrient uptake processes. The lipid content of the control (ASN III medium) was 16%, followed by the ossein effluent comprised of high total dissolved solids (HTDS) 15% and low total dissolved solids LTDS 17%, respectively, which implies the relative lipid production of the strain grown in the effluent. The fatty acid profile of *Chlorella vulgaris* BDU G91771 in the selected effluent condition indicated its suitability for biodiesel production.

Keywords: *Chlorella sp.,* Ossein effluent (HTDS and LTDS), Sterilized and unsterilized ossein effluent, Lipid and fatty acids, Biodiesel.

1. INTRODUCTION

Escalating demand for fossil fuels and the increasing emission of carbon dioxide into the atmosphere has necessitated scientists to focus on alternative biomass-derived fuels the world over. One promising source of biomass for unconventional fuel production is microalgae that can grow rapidly in minimal nutrients and synthesize and accumulate large amounts (20%-50% of dry mass) of neutral lipid [1].

The critical starting point for this process is identifying suitable algal strains that possess high constituent amounts of total lipids. Few earlier attempts of large-scale cultivation trials were unsuccessful due to low lipid production under outdoor culture conditions. Strains with high growth potential and augmented higher lipid productivity rate with the versatility to adapt and grow in various environmental conditions are of prime importance.

For any microalgal cultivation, the water footprint and inorganic nutrients are expensive. Besides, though found highly stimulatory for microalgal growth, organic carbon increases the feedstock cost [2]. Thus, an economically acceptable and environmentally sustainable nutrient source for alga-based biodiesel is the need of the hour. One of the crucial aspects of commercial biodiesel production is to decrease the usage of water and nutrients footprint. Algae can be successfully cultivated in seawater and wastewaters, which will be a great source to reduce water footprint. Thus the promising approach is to grow higher lipid-producing algae in view of biodiesel production coupled with wastewater treatment will gain significance. Besides, microalgae cultivation in wastewater can effectively remove the surfeit nutrients and fix the carbon from the atmosphere by photosynthesis activity. They consume nitrogen and phosphorous from the effluent. Therefore, cultivating marine microalgae in the effluent may also serve as an alternative sustainable approach to reduce the nutrient footprints coupled with lipid production.

Cultivating microalgae in swine wastes, dairy manure, and other animal residues has been reported [3] where only a few reports represent experiments using ossein outlet [4]. Ossein discharge produces a considerable volume of opulent calcium effluent into two major streams (i) low total dissolved solids (LTDS) and high total dissolved solids (HTDS) containing calcium

phosphate (DCP). Ossein effluent has rich organic nitrogen, minerals, and trace elements, making the appropriate nutrient inputs for microalgal cultivation [5]. Thus, the study aims to screen the potential strain from various geographical niches of India varying in growth and lipid productivity with a view to look at its possibility for biodiesel. Microalgae has the potential to grow in heterotrophic and mixotrophic nutritional mode in addition to autotrophic mode. Hence, the work targets to evaluate the growth of the organism in ossein effluent (wastewater water discharge during the process of decalcification of cattle bones), namely low total dissolved solids (LTDS) and high total dissolved solids ossein (HTDS), as an alternative economic nutrient base in view of economical and sustainable biodiesel approach.

2. MATERIAL AND METHODS

2.1. Selection of strains

A total of three cyanobacteria namely *Phormidium valderianum* BDU 20041, *Oscillatoria willei* BDU 130791, *Spirulina subsalsa* BDU 141201; and microalgae consisting of three *Chlorella sp.* namely BDUG91771, BDU G20021, BDU G3006, *and a Picochlorum sp.* BDU G10024 was obtained from the repository of the National Facility for Marine Cyanobacteria (*Sponsored by DBT, Govt of India*) for the study. All the chosen marine strains belong to various geographical Indian coastal niches namely, Tuticorin, Tiruchendur, Cuddalore, Marakkanam, Point Calimere, and Kakinada Port. The strains were selected based on their rapid growth and the passport data particulars maintained in the repository.

All the selected strains were observed under an inverted light microscope (Leica DMI 3000B) for morphological and unialgal conformity following standard taxonomic keys [6].

2.2. Growth maintenance

The selected strains were grown in ASN III marine medium [7] in Erlenmeyer flasks under continuous illumination using white fluorescent light at 1500 Lux light intensity at 25±2°C in a controlled condition and maintained in the repository of National Facility for Marine Cyanobacteria (NFMC), sponsored by Department of Biotechnology (DBT), *Govt. of India*. A volume of 5ml, mid-log phase uniform culture at 7thday was harvested by centrifugation at 5000Xg and used as inoculum for experimental studies.

2.3. Source of the effluent

Ossein effluent (HTDS and LTDS) were obtained from the clarification units during the gelatin production system from Pioneer Jellice Industries, SIPCOT Cuddalore, Tamil Nadu, India. The effluent differed in amounts of the total dissolved solids (TDS), hence named as high TDS (HTDS) and low TDS (LTDS). The collected effluent was stored in black plastic cans at 4°C to avoid microbial growth till use. Both sterile and unsterile (HTDS and LTDS) effluent were used for further studies. The best-chosen strain was evaluated for growth and high lipid production in ossein effluent.

2.4. Screening for potential strain and experimental conditions

The selected seven strains belonging to both marine cyanobacteria and microalgae were inoculated in ASN III medium and evaluated for growth parameters and lipid productivity.

The chosen best strain was then inoculated in both sterilized and unsterilized HTDS and LTDS ossein effluent to evaluate the growth and lipid productivity for the period of seven days. A known volume of culture was harvested by centrifugation and estimated for its growth at regular intervals and at 7th day as endpoint lipid production. Organism grown in ASN III medium served as control. A known volume of culture was centrifuged at 5000 g rpm and growth in terms in terms of chlorophyll-*a* as biomass component was estimated by extracting the pellet in methanol (80%) following by dark incubation at 4°C, and the clear supernatant, was observed under optical density read at 663 nm (Cary 100 bio UV-Vis Spectrophotometer), following the protocol [8]. Similarly, a known volume culture was centrifuged at 5000g rpm, and the dry cell weight (gL^{-1}) was calculated by washing the pellet twice with distilled water followed by drying at 60°C. The dried biomass was weighed until two concordant values [4].

The strains selected for the study were also estimated for their lipid productivity [9] and fatty acids [10] analysis. In all the aforementioned analysis, ASN III medium served as control.

2.5. Lipid extraction and quantification

At endpoint, growth organisms were extracted for total lipid using a binary solvent system described [9]. The solvent mixture of chloroform: methanol (2:1) was added to dried algal biomass, and repeated the extraction process until lipid was extracted completely.

Water was added to the crude lipid, and the lower layer-comprising lipid was carefully collected. The moisture content of the collected lipid was eliminated by passing it through the sodium sulfate bed. Solvent containing lipid was evaporated in the rotary evaporator (Evator II), and total lipid was measured gravimetrically.

2.6. Fatty acid profile analysis

Lipids extracted from the organisms were transesterified by a modified two-step process [10]. Initially, the lipid was saponified with 3.75M NaOH at 100°C for 30 min, and subjected to methanolysis using 5% methanolic hydrochloride at 80°C for 20 min.

Methanolysed contents were then cooled, and a mixture of freshly prepared hexane: diethyl ether (1:1) was added and vortexed for 1 min, allowed to stand for 10 min. to enable phase separation. The aqueous phase was carefully discarded, and the hexane phase containing methylated fatty acids was washed with 300 mM sodium hydroxide. The fatty acid methyl esters (FAME) obtained were analyzed using Clarus 500 gas chromatograph (PerkinElmer, USA) equipped with a flame ionization detector using SP 2650 (100m) column (Supelco St. Louis, MO, USA). The carrier gas used was helium. The temperature at the injector port and detector was 260°C. The initial column temperature was set at 140°C for 1 min and increased to 260°C at the rate of 4°C min. The resultant peaks were noted and compared with the standard fatty acids.

2.7. Statistical analysis

All experiments were carried out in triplicates and the data expressed is an average of mean with S.D. using Origin 8 software (Origin Lab Corporation).

3. RESULTS AND DISCUSSION

Effluent mediated microalgal growth for biofuel production offers twofold advantages (i) microalgae remediate wastewater by removal of nitrogen, phosphorus, and other inorganic nutrients, with their ability to grow under limited conditions and cost-effective nutrient inputs (ii) microalgae synthesize and accumulate large quantities of neutral lipids (20-50 % dry weight of biomass) under nutrient limiting conditions, which is an added advantage in view of effluent based growth [11].

3.1. Screening of potent strain for lipid productivity

Fast-growing seven strains belonging to different regions from the National repository for marine cyanobacteria (Sponsored by DBT, Govt. of India) were examined for their morphology under an inverted light microscope (Leica DMI 3000B), and the micrographs were shown in Fig.1.

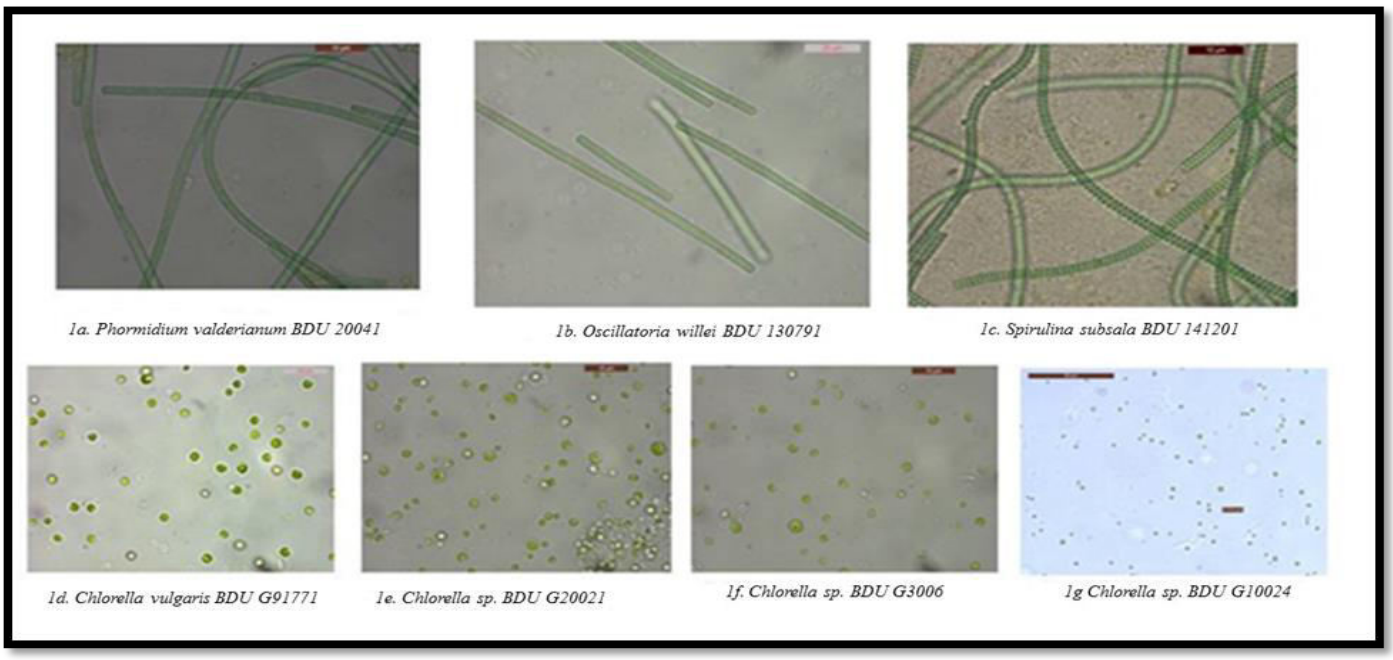


Fig. 1: Light microscopic pictures (40X) of marine cyanobacteria and microalgal strains selected for growth and lipid production.

The passport data of the selected strains in the repository are provided in Table 1. Of the selected strains, three belong to Cyanophyte namely, *Phormidium valderianum* BDU 20041, *Oscillatoria willei* BDU 130791, *Spirulina subsalsa* BDU 141201 and the other four strains of microalgae belonging to Chlorophyte members namely, *Chlorella vulgaris* BDU G91771, *Chlorella sp.* BDU G20021, BDU G3006, and *Picochlorum sp.* BDU G10024 (Fig. 1a-1g) were identified with their unique morpho-logical traits mentioned in the taxonomic keys [6].

In the study, Table 1 represents the selection of strains from various geographical niches stretching from coastal regions of the mainland of India. The strains passport details from the repository were geographically tagged with the unique identification number. According to scientists [12, 13], India is one of the marine diversity hotspots. The geographical location is the prime factor in choosing the best potential strain for growth coupled with lipid production, in line with the study approach.

Table 1: **Passport data of the selected marine cyanobacteria and microalgal strains selected for growth and lipid production**

Strains	Place of isolation	GPS data
Phormidium valderianum BDU 20041	Point calimere	10°29'93.26277 N, 79° 84' 56.0093 E
Oscillatoria willei BDU 130791	Kakinada Harbor	16° 59' 20.6340" N and 82° 14' 50.8812" E.
Spirulina subsalsa BDU 141201	Marakkanam	12° 11' 13.0272" N and 79° 55' 40.4220" E.
Chlorella vulgaris. BDUG91771	Tuticorin	8° 45' 32.64'' N 78° 08' 09.13'' E
Chlorella sp. BDU G20021	Cuddalore	12° 16' 29.34'' N 79° 56' 31.60'' E
Chlorella sp. BDU G3006	Marakkanam	12° 12' 32.53'' N 79° 56' 11.16'' E
Picochlorum sp. BDU G100241	Tiruchendur	8° 28' 58.04'' N 78° 05' 32.42'' E

Among cyanobacteria, it is reported that filamentous forms especially *Phormidium sp, Oscillatoria sp and Spirulina sp* are attempted for bio prospecting studies in several perspectives [4, 14, 15]. Also, cyanobacteria are potent enough to tolerate varied types of effluent and nutrient-limited conditions [16-19]. Similarly, *Chlorella sp.* was reported as an ideal candidate for high lipid productivity [20]. Thus the study utilizes the rich coastal wealth and aims to screen one potential strain insight to rapid growth coupled high lipid productivity in view of environmental concern and economic feasibility for biodiesel perspective.

3.2. Growth coupled with lipid production

Endpoint growth on the 7th day was measured in terms of chlorophyll *a* and dry weight as a biomass component. Among the seven selected strains tested for their growth, *O. willei* BDU130791 (9.8µg) representing cyanobacteria and *Chlorella sp.* BDU G3006 (13.3µg) representing microalgae produced maximum biomass both in terms of chlorophyll *a* (Table 2). *Spirulina subsalsa* BDU 141201 (1.07g) representing cyanobacteria and in microalgae *Chlorella sp.* BDU G3006 (0.36g) ranked higher in terms of dry weight. All other tested organisms yielded lesser biomass represented in table 2.

Chlorophyll *a* is the essential component of the photosynthetic unit which participates in the light-harvesting process and activates the photosynthetic apparatus. Similarly dry biomass also increases with the chlorophyll, when growth is in log phase [21].

Equally, lipid productivity was estimated on the 7th day, and the production rate was of the order *C. vulgaris.* BDU G91771> *Picochlorum sp.* BDU G100241> *Spirulina subsalsa* BDU 141201> *Oscillatoria willei* BDU 130791> *Chlorella sp.* BDU G3006> *Phormidium valderianum* BDU 20041> *Chlorella sp.* BDU G20021. Of all the strains tested, *Chlorella vulgaris.* BDUG91771 ranked higher with 16% lipid, which is reasonably high for biodiesel production (Table 2).

From the results, it could be inferred that *C. vulgaris.* BDU G91771, even though it divulged slightly lesser growth, exhibited higher lipid productivity. The other strains, namely *O. willei* BDU130791, *Chlorella sp.* BDU G3006, though shown rapid growth, their lipid producing capacity was comparatively less, representing that algal biomass competes for photosynthetic assimilates and thereby altering its growth and stimulating lipid biosynthesis. As a cause, environmental stress and photo-oxidative damage lead to enhanced lipid production. This phenomenon of microalgae substantiates the mediocre growth with high lipid production, suitable for biodiesel production. Hence *C.*

vulgaris BDU G91771 in spite of moderate growth coupled with high lipid yield was chosen for further experiments (Table 2). According to scientists [20], *Chlorella* sp. is one of the promising marine microalgae employed to accumulate high amounts of lipids or carbohydrates under stress conditions, and, for this reason, it is of interest in biofuel production. High production costs and energy consumption are associated with its mass cultivation which is still in tuning. Thus, the present study attempts to reduce costs and environmental impact of *Chlorella* by cultivating it in an economical and environmental malleability process.

Table 2: Growth parameters of the selected strain in ASN III medium on 7th day at the pH: 7.2, Temperature: 25±2°C; Light Intensity: 1500lux

Growth parameters	*Phormidium valderianum* BDU 20041	*Oscillatoria willei* BDU 130791	*Spirulina subsalsa* BDU 141201	*Chlorella vulgaris.* BDU G91771	*Chlorella sp.* BDU G20021	*Chlorella sp.* BDU G3006	*Picochlorum sp.* BDU G10024
Chlorophyll a (µg)	5.07	9.83	8.24	10	12.5	13.3	11.2
Dryweight (mg)	0.65	0.92	1.07	0.2	0.3	0.36	0.15
Lipid (%)	6.8	12.9	13.2	16	6	9	14.5

3.3. Effluent mediated growth for lipid productivity

The chosen best strain; *C. vulgaris* BDU G91771, was grown in ossein effluent to remediate the same by utilizing it as a nutrient source for lipid productivity. In particular, ossein (decalcified bone) effluent contains the chief organic substance of the animal bone tissue obtained as a residue in the clarification process of the gelatin production system. The considerable volume, pungent odor, and high organic and inorganic contents are of significant concern in the disposal of the ossein effluent [22]. The chosen *C. vulgaris* BDU G91771 strain was grown in both sterilized and unsterilized HTDS and LTDS effluent. Growth as biomass component was measured in terms of chlorophyll '*a*' and dryweight.

The criteria for choosing the unsterilized effluent (HTDS and LTDS) condition are less feasibility to sterilize the wastewater because of the use of enormous volume in large-scale cultivation. Also, it is reported [23] that in reality, the effluent in nature consists of a consortium of the different microbial communities (bacteria and fungi), making the mixotrophic condition amenable for cultivation for microalgae.

Thus, the chosen marine microalga *C. vulgaris* BDU G91771 cultivated in three independent modes namely (i) autotrophic (ASN III medium), (ii) heterotrophic (sterilized ossein effluent) and mixotrophic (unsterilized ossein effluent) had almost similar growth efficiency.

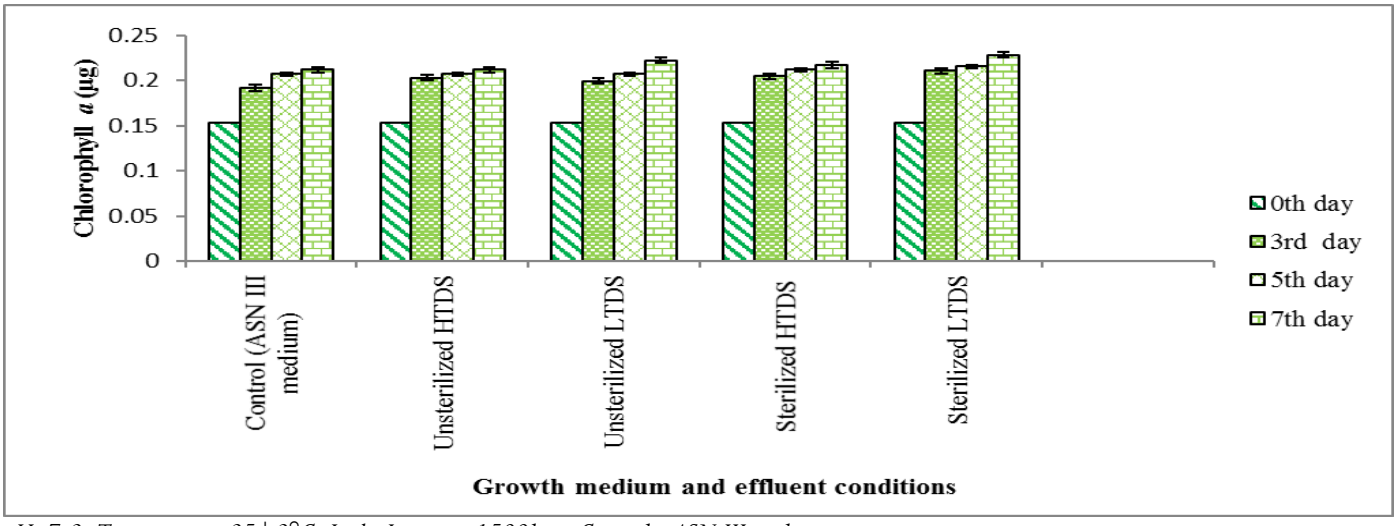
When the growth was compared between the HTDS and LTDS effluent, chlorophyll *a* was slightly higher in both sterilized and unsterilized LTDS effluent (Fig. 2). Increased growth in LTDS effluent can be attributed to lesser particulate matter, promoting photosynthetic efficiency because of higher light penetration, thereby supporting the microalga's growth. The same phenomenon of growth was observed with the dryweight as biomass component in both sterilized and unsterilized (HTDS and LTDS) effluent **(**Fig.3).

Interestingly, the organism demonstrated a moderately equivalent growth pattern in control (i.e) artificial synthetic seawater medium (ASN III medium) which is in line with the effluent. From Fig. 2 and 3, it could be inferred that the organism can utilize the organic and inorganic chemicals and grow in both HTDS and LTDS ossein effluent.

In connection to wastewater treatment concerning algae, bacteria indeed support the photoautotrophic growth of microalgae by providing CO_2 through their heterotrophic mode mineralizing into inorganic compounds for the microalgae to uptake. In return, microalgae provide O_2 required by the bacteria to degrade organic matter in the dark respiration process. Similarly, Wang et al. [24] reported photosynthesis by a microalgal consortium to generate a surplus volume of dissolved oxygen to support the nitrification process, a prime cellular metabolic process. This report was analogous with Huang et al. [25] demonstrated with *C. vulgaris* UTEX 25 and *Auxenocholrella* sp. in mixotrophic conditions. Henceforth this process can meet the energy need for the system and can be one key factor in life cell assessment.

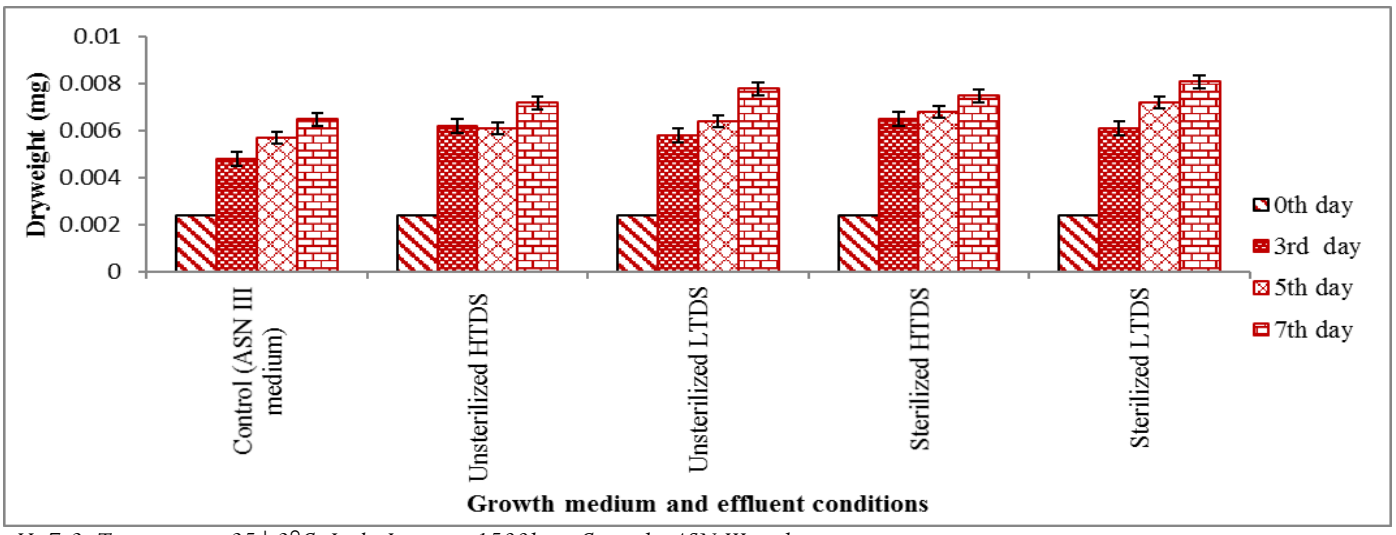
Ossein effluent is rich in organic content and inorganic ions, and its treatment is eye catchy as it is rich in nutrients including nitrogen, phosphorus, calcium, chloride and total organic carbon like amino and fatty

acids. High nutrient content makes them efficient as wastewater substrate for microalgal cultivation to achieve reasonably high biomass yield for energy generation [26, 27].



pH: 7.2, Temperature: 25±2°C; Light Intensity:1500lux. Control - ASN III medium

Fig. 2: Growth of *C. vulgaris* BDU G91771 in terms of Chlorophyll *a* in unsterilized and sterilized ossein effluent (HTDS and LTDS)



pH: 7.2, Temperature: 25±2°C; Light Intensity:1500lux. Control - ASN III medium

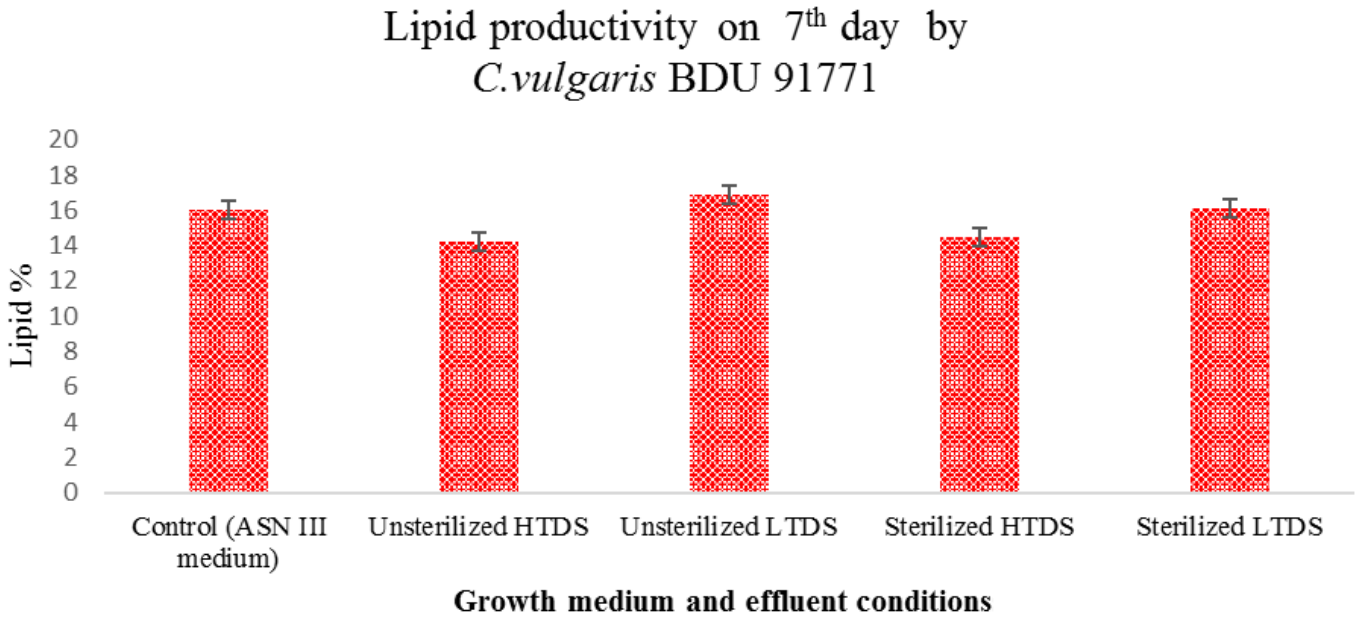
Fig. 3: Growth of *C. vulgaris* BDUG91771 in terms of dryweight in unsterilized and sterilized ossein effluent (HTDS and LTDS)

Nutrient removal (nitrate and phosphorous) is another advantageous parameter in wastewater cultivation and is attributed to photosynthetic activity and biomass production. The mechanism of total nitrate and phosphate assimilations in the effluent are presumed to be biomass uptake in the exponential growth of the organism. Therefore, systematic optimization of different consortia of nutrients from various water resources to accomplish high lipid and desirable fatty acid production is imperative in sustainable biodiesel production [28]. Based on growth of *C. vulgaris* BDU G91771 in ossein effluent (in sterilized and unsterilized conditions), beyond doubt demonstrates the organism's mixotrophic potentiality.

Henceforth, the chosen strain *C. vulgaris* BDU G91771 was estimated for the lipid production in all the autotrophic, heterotrophic and mixotrophic mode in view of biodiesel approach.

3.4. Lipid Productivity

The selected strain *C. vulgaris* BDU G91771 was estimated for lipid production in both the control and effluent conditions as it attains a progressed growth. Interestingly, a maximum of 17% lipid was observed in LTDS effluent, followed by HTDS (15%) and control ASN III medium with 16% (Fig. 4). Of all the tested conditions, unsterilized LTDS effluent exhibited maximum lipid productivity of 16.9% (Fig. 4). Considering the economic feasibility, ossein effluent was used instead of laboratory-grade nutrients required for microlagal growth. Also in the present study, the growth and lipid were comparatively similar in ASN III medium (control), and the effluent tested which implies, the nutrient equivalency in both the categories. This mixotrophic lipid production in the unsterilized effluent condition substantiates the finding [29], where the metabolic machinery of microalgae turns on or off to biochemical synthesis components with respect to major nutrient sources namely, nitrogen and phosphorous. When there is an adequate nutrient (N and P) in the medium (ossein effluent), the growth gets stimulated, incorporating nitrogen towards protein synthesis and sacrificing by the lipid production. Besides, it is reported that carbon-rich compounds accumulate in the organism under nutrient-deprived conditions when carbon and energy are much higher than required [30]. The mechanism of lipid production under effluent mediated growth by *C. vulgaris* BDU G 91771 substantiates that CO_2 concentrating mechanism acquires and fixes CO_2 from ambient environment and convert into lipid and carbohydrate together with the low photosynthetic activity in mixotrophic conditions. This phenomenon could be substantiated in the study that ossein effluent is rich in calcium and it is leached during the calcification process through the carbon concentrating mechanism obtained from the effluent's enriched CO_2 [5]. All these metabolic processes conclude the optimum lipid production state in the ossein effluent. The lipids obtained in *C. vulgaris* BDU G91771 from the best-chosen effluent condition (unsterilized LTDS) and the control medium were further analyzed for fatty acid methyl ester (FAME) analysis to test its suitability for biodiesel.



pH 7.2, Temperature 25±2°C; 1500lux. Control - ASN III medium

Fig. 4: Percentage lipid of *C. vulgaris* BDUG91771 on 7th day in unsterilized and sterilized ossein effluent (HTDS and LTDS)

3.5. Fatty acid composition

The identified high lipid yielding strain *C. vulgaris* BDU G91771 was further characterized for their fatty acid profile in ASN medium (control) and unsterilized LTDS effluent. The fatty acid profile varied inevitably in the effluent conditions tested. According to Mathimani et al. [20], the quality and quantity of fatty acids is a critical parameter for biodiesel quality. The selected strain showed the predominance of middle and long chain fatty acid, namely palmitic (C16), palmitoleic (C16:1),

cis 10- heptadecenoic acid (C17:1), stearic (C18:0), oleic (C18:1n9c), linoleic acid ((C18:3n3), in both the control and effluent (unsterilized LTDS) grown samples. The total fraction of the fatty acid methyl esters (FAME) extracted, and the specific composition of the individual detected FAME are shown in table 3. The concentration of palmitic and linoleic acid was found to be high in the tested LTDS effluent.

Table 3: Fatty acid profile of *C. vulgaris* BDUG91771 in the ASN III medium and unsterilized LTDS ossein effluent

SI. NO	FATTY ACIDS	ASN III medium (control)	Unsterilized LTDS ossein effluent
1	Caprylic acid (C8:0)	0	1.3
2	Capric acid (C10:0)	0.95	1.4
3	Undecanoic acid (C 11:0)	0	0.0
4	Lauric acid (C12:0)	0	2.3
5	Tridecanoic acid (C13:0)	0	0.1
6	Myristic acid (C14:0)	1.29	2.3
7	Myristoleic acid (C14:1)	0	1.3
8	Pentadecanoic acid (C 15:0)	0	2.3
9	*cis* 10 Pentadeconoic acid (C 15:1)	9.39	0.0
10	Palmitic acid (C 16:0)	10.59	22.9
11	Palmitoleic acid (C16:1)	7.92	19.4
12	Heptadecanoic acid (C17:0)	0	0.0
13	*cis* 10 heptadecenoic acid (C17:1)	12.37	1.1
14	Stearic acid (C18:0)	11.57	1.3
15	Oleic acid (18:1)	13.67	16.2
16	Linolelaidic acid (C 18:2t)	0	0.3
17	Linoleic acid (C 18:2c)	1.7	20.63
18	Gamma linolenic acid (C 18:3)	0	12.8
19	Heneicosanoic acid (C 21:0)	0	1.3
20	*cis* 11,14 eicosadienoic acid (C 20:2)	0	1.4
21	*cis* 8, 11, 14- Eicosatrienoic acid (C 20:3)	0	1.3
22	Erucic acid (C 22:1)	0	0.3
23	Arachidonic acid (C 20:4)	0	1.4
24	*cis* 13,16 docosadienoic acid (C 22:2)	0	0.0
25	Tricosanoic acid (C23:0)	0	2.6
26	*cis* 5,8,11,14,17 eicosapentaenoic acid (C 20:5)	0	2.6
27	Lignoceric acid (C 24:0)	0	0.0
28	Nervonic acid (C 24:1)	0	0.0
29	Docosahexaenoic acid (C 22:6)	0	2.3
30	Unidentified	11.1	14.96
31	Saturated fatty acids (SFA)	24.88	28.07
32	Monounsaturated fatty acids MUFA's	22.07	38.269
33	Polyunsaturated fatty acids PUFA's	15.16	23.822

The key factors determining the biodiesel qualities are (i) fatty acid chain length, (ii) degree of unsaturation (regulates cetane number and cold properties), (iii) oxidative stability, (iv)viscosity [31]. Generally, palmitic acid (C16:0) and oleic acid (C18:1), are the imperative fatty acid classes that decide the fuel properties [32], which correlates with the present finding. The striking finding in the present study is that the organism in LTDS effluent possessed increased C16:0 fatty acid over the control (ASN III media), indicating the nutrient-deficient condition has relatively enhanced the 16:0 fatty acid production. Furthermore, the phenomenon behind this is the overexpression of AMP deaminase which aids as a precursor of acetyl CO-A for fatty acid biosynthesis in the hostile condition [12, 20] which emulates in the present findings.

The present study gives us a lead that marine microalga *C. vulgaris* BDU G91771 could be cultivated in ossein

effluent in view of biodiesel approach addressing the water footprint and liquid waste treatment, which is economical and environmentally friendly.

4. CONCLUSION

The novelty of the study is identifying an ossein effluent as growth medium for marine isolate *C. vulgaris* BDU G91771. Growth of this strain in LTDS ossein effluent will be ecofrindly, economical and will reduce waterfoot print. The organism's growth potential coupled with lipid and fatty acid content is a viable option for biodiesel production and could be a promising strain to be a part in Indian National biofuel policy.

5. ACKNOWLEDGEMENTS

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Conflict of interest

Authors declare no conflict of interest in the manuscript.

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