JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

"Exploring Oleaginous Yeasts from Diverse Environmental Sources for Biodiesel Production Using Crude Glycerol: Isolation, Characterization, and Lipid Analysis."

[1]Ms Ayesha Shaikh, [2]Dr Arjumanara Surti,

[1]PhD Scholar, [2]Head, Associate Professor
[1]Microbiology Department,
[1]Sophia College for Women (Autonomous), Mumbai, India

Abstract: Over the past few years, there has been a surge in interest in microbial lipids as potential raw materials for biodiesel production. Oleaginous yeast species are being considered as an alternative for the production of lipids or triacylglycerides (TAGs). These yeasts are usually non-pathogenic and have the potential to store high amounts of TAGs ranging from 20% to 80% of their cell weight depending on the culture conditions in the form of lipid bodies in the cell. The utilization of industrial by-products as carbon sources is a viable option for low-cost lipid fermentation with oleaginous microorganisms on a large scale. The present study aimed to screen oleaginous yeast strains from diverse environmental sources and select the strain that accumulated the largest quantity of lipids using crude glycerol as a carbon source and corn steep liquor (CSL) as a nitrogen source. GC-MS analysis showed that the procured crude glycerol sample from a biodiesel plant in Maharashtra has 70.2% glycerol. Through the initial screening process, a total of 35 yeast strains were isolated from different environmental samples using pure glycerol as a carbon source, 29 yeast strains were determined as oleaginous yeast by Sudan Black B staining. Sudan Black B staining revealed the presence of lipid inclusion bodies in the cells during cultivation under nitrogen-limiting conditions. The lipid content of these 29 oleaginous yeast strains was then compared in a crude glycerol-based medium having a C: N ratio of 10:1. The result indicated that selected 29 oleaginous yeast strains can grow on crude glycerol and produce lipids that can be used as raw materials for the production of biodiesel. The oleaginous yeast (IS001) isolated from Dockyard seawater showed the highest lipid accumulation reaching 64.61% of lipid content. C16:0, C18:0, C18:1, and C18:2 essential fatty acids for biodiesel production were detected by GC-MS in the lipid accumulated by this strain. This study provides a scientific basis for adopting crude glycerol as an effective carbon source for lipid production which will reduce costs and promote waste recycling.

Keywords: - Crude glycerol; Lipid; Oleaginous yeast; Sudan Black B; GC-MS; Biodiesel.

I. INTRODUCTION

In the last few years, the utilization of fossil resources and declining of various petroleum reserves has affected the environment immensely due to the emission of CO_2 (1). Sustainable and cost-effective routes for the renewable production of chemicals and fuels are needed to support the growing population and economy with a reduced carbon footprint. Among these alternatives, biofuels have emerged as promising candidates due to their potential to mitigate greenhouse gas emissions and reduce dependency on non-renewable energy sources. In particular, microbial-derived biofuels, such as those produced by oleaginous microorganisms, offer unique advantages owing to their high lipid content and rapid growth rates.

Among biofuels, biodiesel is considered to be one of the most sustainable and renewable substitutes for fossil diesel fuel. Biodiesel is considered to be a bio-based analog since it is a renewable fuel and environmentally friendly. The first-generation biodiesel obtained from various edible feedstocks such as sugar, starch, and oil crops was proposed, to ensure environmentally

friendly fuels. Even though it was efficient some concerns surrounded it such as the overall carbon footprint, environmental damage, and the food versus fuel competition. Therefore, second-generation biodiesel was introduced to overcome the drawbacks of first-generation biodiesel. In this case, alternative feedstocks such as non-edible feedstocks like waste vegetable oils and fats, and non-food crops are being used. However, this has ended up demanding renewable standing substrates majorly relying upon plant-based resources.

Food-grade oil extracted from various edible plants such as rapeseed, sunflower, soybean, or palm is most commonly used as feedstocks for biodiesel production. However indiscriminate use of edible plant oils for biodiesel production has raised concerns regarding their necessity as food ingredients. Also, an increase in the cost of edible plants has affected both the economics of food and biofuel production. Microbial oils/lipids are therefore proposed as an alternative to vegetable oil or animal–fat-based feedstocks.

Microorganisms are known to be natural oil producers due to their ability to accumulate more than 20% w/w of lipids on a cell dry weight, such organisms are called oleaginous microorganisms (OM) (2). These microbial lipids have the potential to be used as a raw material for the production of biodiesel by transesterification. Oleaginous microorganisms include microalgae, bacteria, fungi, and yeasts.

An interesting class of oleaginous microorganisms is oleaginous yeast. Oleaginous yeast has the potential to store high amounts of triglycerides in the form of lipid bodies in the cell. This lipid accumulation is primarily driven by an excess of carbon sources with limited availability of other nutrients, such as nitrogen (3). Yeast-derived lipids, primarily triacylglycerols, share similar chemical compositions with lipids obtained from plant oilseeds, making them comparable in terms of their applications. Moreover, yeasts exhibit versatility in utilizing a wide range of nutrient sources, including industrial wastes, which can potentially reduce production costs.

Crude glycerol (CG), a byproduct of the biodiesel industry, is generated during the transesterification of vegetable oils. However, CG presents challenges as it contains methanol, soap, and ash, making it highly problematic (4). Crude glycerol in large amounts can pose a threat to the environment. Therefore, there is a need to convert this crude glycerol into value-added products using biotechnological processes, which brings new revenue to biodiesel producers. Considering this, when yeast lipid is utilized as a substrate in transesterification, glycerol is produced alongside biodiesel. Consequently, the resulting waste/ crude glycerol can be recycled by employing it as a carbon source for the growth and lipid accumulation of oleaginous yeasts once again. This cyclical process can be perpetuated indefinitely to fully exploit the potential of both yeast lipid and glycerol (5).

In biodiesel production, certain types of lipids are particularly significant, notably those rich in long-chain fatty acids such as linoleic acid and oleic acid (6). These fatty acids are commonly found in triglycerides present in various feedstocks used for biodiesel production, such as vegetable oils and animal fats. Therefore, in lipid analysis using GC-MS for biodiesel applications, the identification and quantification of triglycerides containing these key fatty acids, along with other relevant fatty acid compositions, are essential (7). By assessing the abundance and distribution of these fatty acids, GC-MS analysis provides critical insights into the suitability of lipid feedstocks for biodiesel production and enables optimization of the production process to enhance biodiesel yield and quality (8).

Oleaginous yeasts are prevalent in diverse natural habitats, including soils, leaves, and flowers, as well as various environmental niches such as seawater and mangrove forests (9). The objective of this study was to isolate and screen for potential oleaginous yeasts sourced from different environmental samples capable of utilizing crude glycerol as a carbon source. Additionally, the investigation extends to analyzing the lipid content of a selected yeast isolate, further enhancing our understanding of its lipid-producing capabilities and identifying promising candidate/s for lipid production with potential applications in various industrial sectors such as biodiesel production.

Materials and Method:

A. Enrichment and Isolation of yeast from various samples:

One gram of each sample (soil sample from the petrol pump and mangrove forest in Mumbai; fermented fish, fruit waste, coconut waste, sugarcane bagasse, pickle, spoiled dates collected from various markets across Mumbai and Navi Mumbai) and one ml of water sample (from Dockyard road, Haji Ali, Mithi river and Used oil from a restaurant in Mumbai) were added separately to glycerol enriched medium containing - [(100 g/L glycerol, 1 g/L $(NH_4)_2SO_4$, 1g/L KH_2PO_4, 0.5 g/L $MgSO_4.7H_2O$, and 0.2 g/L yeast extract), and 3.3 ml of streptomycin (to prevent contamination from bacteria and fungi 10,000 units/ml)] pH 6, and were incubated on a shaker at 28 °C for 24-72 hours with constant shaking at 150 rpm (10). All the chemicals used in the study were of analytical grade.

From this enriched medium, culture dilutions were made and plated onto the glycerol media of the above composition and incubated at 28°C for 48 hours. All the isolates obtained were subjected to microscopic observation (Gram staining) to observe for budding characteristics to confirm as yeast. The isolated colonies of yeast were then purified further and maintained on glycerol agar slants.

B. Screening for oleaginous yeasts:

The isolated colonies of yeasts were further screened for their lipid-producing abilities by qualitative analysis with the Sudan Black B staining technique (11). The potential oleaginous yeast colonies that showed the presence of lipid globules were maintained and subcultured on glycerol slants and stored at 4°C until use.

C. Cultivation of the yeast isolate/s in a medium containing waste (crude) glycerol as a carbon source, and corn steep liquor (CSL) as a nitrogen source:

The oleaginous yeast identified through Sudan Black B staining was cultivated in a medium using crude glycerol as a carbon source and CSL as a nitrogen source. The crude glycerol that was used in the present study was a by-product of a biodiesel production plant located in Nashik (Maharashtra). Before the crude glycerol can be considered for its utilization as a carbon source for the growth of the oleaginous yeast it is necessary to characterize the crude glycerol for its physical, and chemical properties. The characterization of crude glycerol involved assessing its color, density, pH, soap concentration, ash content, and glycerol content (12).

1. Color: The color of the procured crude glycerol obtained from the biodiesel industry was noted.
2. Density and pH: The weight of 2 ml crude glycerol was measured at room temperature. The density of the crude glycerol was determined by dividing the weight by the volume (2ml). To determine the pH, 1 ml of the crude glycerol was dissolved in 50 ml of deionized water and the pH of the solution was measured at room temperature using a digital pH meter.
3. Soap concentration: 0.5 ml of crude glycerol was dissolved in 100 ml of acetone containing 2% (v/v) distilled water. This solution was titrated against 0.1 M HCl using 1% (w/v) phenolphthalein as an indicator until the red color of the solution disappeared. The volume of the titrate that was consumed in this step was designated as "A". Once the solution became colorless, 1ml of bromophenol blue indicator (0.4% w/v) was added to neutralize the solution, and the titration was continued using 0.1 M HCl until the solution changed the color from blue to yellow. The volume of the titrate that was consumed in this step was designated as "B". The soap concentration was calculated using the following formula:

$$\% \text{ Soap (sodium oleate)} = \frac{\text{Volume of titrant "B" (ml)} \times 0.001 \times 0.1 \times 304.04 \times 100}{\text{Amount of sample taken (g)}}$$

4. Ash content: 10 ml of crude glycerol sample was heated at 750 °C for 3 hours. After cooling at room temperature, the residue (W1) was weighed using analytical balance until three constant weights were obtained, ensuring accurate biomass measurement, and the ash content was calculated using the following formula (W_1 / 10 x 100%).
5. Glycerol concentration: The glycerol content in crude glycerol was determined via Gas Chromatography-Mass Spectrometry (GC-MS).

Preparation of inoculum: A saline suspension of the oleaginous yeast isolates was added to the glycerol medium of the above composition and incubated on a shaker at 28°C for 24 to 72 hours with constant shaking at 150 rpm.

Inoculation in production medium: From the above medium 10% v/v was transferred to the production medium (100g/L crude glycerol, 1g/L $(NH_4)_2SO_4$, 1g/L KH_2PO_4, 0.5g/L $MgSO_4.7H_2O$, and 0.2g/L CSL), pH 6 and incubated on a shaker at 28°C for 24-72 hours with constant shaking at 150 rpm.

D. Determination of biomass and production of lipids from the yeast isolates:

The above production medium was used for the determination of biomass and lipid content. The biomass of each isolate was estimated by gravimetric method (13). The production media was centrifuged and the separated cells were dried overnight at 60°C, and weighed using analytical balance until three constant weights were obtained, ensuring accurate biomass measurement.

The lipid from the yeast isolates was then extracted, dried, and weighed by following the Bligh and Dyer method (14). The dried cells were washed with 10 ml of 2M HCl solution to lyse the cells and break down cellular structures. Methanol: chloroform: deionized water (10:5:4) was added to the flask containing cells, and the mixture was shaken at 180 rpm for 2 hours to extract lipids. The lower chloroform layer containing lipids was carefully separated and dried at 70°C for 2 hours. The total lipid content was quantified by gravimetric analysis until three constant weights were obtained, ensuring accurate measurement.

The lipid extracted from the isolate demonstrating maximum lipid production was further analyzed using GC-MS (15,16). The isolate that showed the highest lipid content was selected and preserved on glycerol medium slants at 4°C for subsequent analysis and experimentation.

Results and Discussion:

E. Isolation of yeast from various samples:

A total of 35 isolates with the morphology typical of yeast were isolated from different samples such as soil from a petrol pump and mangrove forest, fermented fish, fruit waste, coconut waste, sugarcane bagasse, pickle, spoiled dates, a seawater sample from Dockyard Road, Haji Ali, Mithi river and Used oil from a restaurant in Mumbai.

F. Screening of oleaginous yeast:

All 35 yeast isolates were observed for lipid accumulation inside the cell by using Sudan Black B staining. The lipid accumulation process requires the exhaustion of a nutrient, usually nitrogen, to allow excess carbon to be incorporated into lipids. The yeast isolates that accumulate lipid intracellularly take the stain and show black color lipid granules when observed under a microscope at 100X magnification. Out of 35 yeast isolates tested 29 of them showed the presence of lipid granules. These 29 oleaginous yeast isolates were then grown in a medium containing crude glycerol as a carbon source.

Figure 1: Enrichment of the Sea water sample (Dockyard Road).

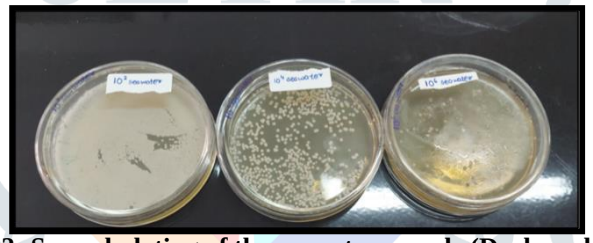

Figure 2: Spread plating of the seawater sample (Dockyard Road).

Figure 1: Gram staining of the isolate IS001

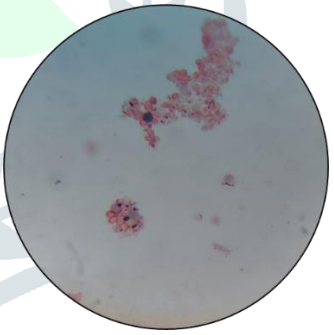

Figure 2: SBB staining of the isolate IS001

G. Characterization of the procured crude glycerol:

Analytical tests were conducted on crude glycerol as it was obtained directly from a biodiesel plant without further processing. Measurements included the determination of the color, density pH, soap concentration, ash content, and glycerol concentration. All the measurements were done in triplicates. The procured crude glycerol was light brown and yellowish in color. The density was found to be 1.2 g/cm^3 which is lower than that of pure glycerol 1.31 g/cm^3 this may be due to the presence of some lighter impurities such as fatty acids methyl esters (FAMEs), fatty acids, methanol, and water in crude glycerol. pH was found to be 6 which was close to that of the pure glycerol which is 6.4, this could be due to the removal of residual alkalis by some post-treatments in the biodiesel plant. The soap content in crude glycerol is related to the character of the feedstock oil used in biodiesel production. If the soap content in the crude glycerol is higher than 14% it will be converted to Free fatty acids (FFA) before being used, since the soap content of the procured crude glycerol was found to be 9.132 % which was less than 14% therefore no conversion to FFA was carried out (17). The ash content was found to be 16%, the ash content in crude glycerol is mainly from the catalyst employed in the transesterification process. The quantification of the glycerol content was achieved using an analytical technique such as GC-MS. The total glycerol content in the crude glycerol was found to be 70.2%. The GC chromatogram for the crude glycerol is represented in Figure 3 and Table 1 shows the results of the GC-MS analysis of crude glycerol.

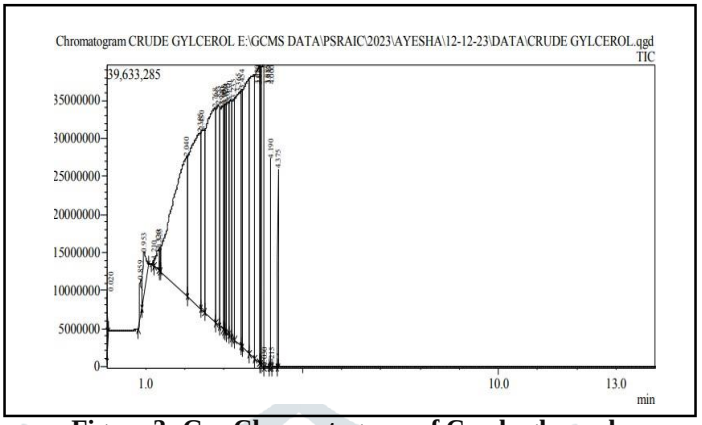

Figure 3: Gas Chromatogram of Crude glycerol

Table 1: Components corresponding to the peaks obtained in the gas chromatogram of crude glycerol

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Name
1	0.020	5327366	0.13	7116434	1-(5-Bicyclo[2.2.1]heptyl)ethylamine
2	0.859	18187647	0.45	5091189	Trimethylsilylmethanol
3	0.953	34957548	0.86	5642481	Phosphinic acid, [(methyl)(formyl)amino]methyl(2-
4	1.210	2233913	0.05	819859	Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid
5	1.320	12872915	0.32	2656964	Allantoic acid
6	1.355	6140915	0.15	3008332	1,3-Dioxolane, 2,4,5-trimethyl-
7	2.040	463031255	11.38	18332691	Phenol
8	2.385	430188346	10.57	23127739	2-Propanone, 1,3-difluoro-
9	2.430	127796874	3.14	23671362	Disulfide, ethyl pentyl
10	2.768	476273397	11.71	28240495	Octanoic acid
11	2.853	171916403	4.23	29001941	Octanoic acid
12	2.940	157164548	3.86	29269563	Atrolactic acid
13	2.995	53424526	1.31	29714465	Ethanethiol, 2-(diethylboryloxy)-
14	3.020	71921567	1.77	29959138	Glycerin
15	3.075	127760834	3.14	30376199	N,N-Dimethyl-O-(1-methyl-butyl)-hydroxylamine
16	3.130	139628589	3.43	30883139	1-(5-Bicyclo[2.2.1]heptyl)ethylamine
17	3.235	132414017	3.25	31641454	Glycerin
18	3.365	314633201	7.73	33051778	3,3-Dimethylpiperidine
19	3.454	91387002	2.25	33987815	N-Methoxy-N-methylacetamide
20	3.630	369741155	9.09	36232912	1,2,3,4-Butanetetrol, [S-(R*,R*)]-
21	3.680	310097813	7.62	36689141	2-Propanol, 1-chloro-3-(1-methylethoxy)-
22	3.885	264463051	6.50	38934107	3-Heptene, 7-(ethylthio)-
23	3.919	82248434	2.02	39229543	Glycerin
24	4.000	165465759	4.07	39705194	Erythritol
25	4.030	1648160	0.04	174189	
26	4.190	18412695	0.45	27545231	Erythritol
27	4.215	1656241	0.04	174462	
28	4.375	17208719	0.42	26175682	Erythritol

H. Determination of biomass and lipid content of the oleaginous yeasts grown in a medium containing waste (crude) glycerol as a carbon source and corn steep liquor (CSL) as a nitrogen source:

Among the 29 oleaginous yeasts, 4 of them (IS001 isolated from seawater dockyard, IS006 and IS007 isolated from fruit waste, IS0015 isolated from a used oil from a restaurant in Mumbai) showed lipid content of more than 40% when grown on a medium using crude glycerol and CSL as carbon source and nitrogen source respectively. Table 2 shows the biomass and lipid content for all 29 isolated oleaginous yeast strains.

It's reported that the best fatty acid composition for biodiesel production is oleic acid (C18:1), linoleic acid (C18:2), palmitic acid (C16:0), stearic acid (C18:0), and linolenic acid (C18:3) (18). The highest lipid content was found in the oleaginous yeast IS001 isolated from the seawater dockyard. The fatty acid composition of this oleaginous yeast was analyzed using GC-MS which is represented in Table 3. There were three dominant fatty acids- linoleic acid (C18:2), oleic acid (C18:1), and palmitic acid (C16:0) of the total identified fatty acids making it suitable for further studies.

Easterling et al. reported that oleaginous yeasts can grow and accumulate lipids when grown on glycerol and have short generation times (19). Also, it is reported that the crude glycerol obtained from the biodiesel contains various elements such as calcium, potassium, magnesium, sulfur, and sodium (20). Therefore, using crude glycerol as a carbon source to produce lipids from oleaginous yeast to be utilized as a biodiesel feedstock would provide a benefit for reducing production costs.

Table 2: Lipid extraction from various samples using the Bligh and Dyer method.

SAMPLE	ISOLATE	CELL BIOMASS Total (g /L)	LIPID WEIGHT (g / L)	LIPID CONTENT (%) Formula (Lipid Content = Lipid Weight / Cell biomass)
Sea Water (Dockyard Road)	IS001	3.25	2.1	64.61 %
Sea Water (Haji Ali)	IS002	5.35	0.5	9.34%
Mithi River (Mahim)	IS003	2.85	0.35	12.28%
Soil Sample (Petrol Pump Parel)	IS004	4.35	0.7	16.09%
Fruit waste (Vashi Market)	IS005	1.57	0.45	28.57%
	IS006	1.30	0.80	61.53%
	IS007	1.60	0.75	46.85%
Soil Sample (Mangrove Forest - Vasai)	IS008	4.80	0.80	16.66%
Fermented Fish	IS009	1.60	0.20	12.59%
	IS010	1.35	0.35	25.92%
Mixed Pickle	IS011	3.60	0.40	11.11%
	IS012	1.60	0.35	21.87%
Peach and Plump waste (Sewri)	IS013	1.50	0.25	16.66%
Used Oil (Restaurant in Bandra)	IS014	4.75	0.12	2.63%
	IS015	2.20	1.05	47.72%
	IS016	4.25	0.40	12.35%
	IS017	2.55	0.90	35.29%
Avocado Fruit waste	IS018	4.55	0.62	13.73%

(Colaba fruit market)	IS019	4.65	1.00	21.50%
Fruit waste (Dadar Fruit Market)	IS020	2.60	0.25	9.61%
	IS021	4.15	0.12	3.01%
Spoiled Grapes (Byculla Market)	IS022	3.05	1.12	36.88%
	IS023	3.85	0.62	16.23%
Spoiled Coconut (Vashi Market)	IS024	3.37	0.12	3.70%
	IS025	5.12	1.37	26.82%
Spoiled Dates (Crawford Market)	IS026	5.75	0.62	10.86%
	IS027	4.67	0.87	18.71%
Sugarcane Bagasse's (Girgaon)	IS028	4.97	1.00	20.10%
	IS029	3.75	1.00	26.66%

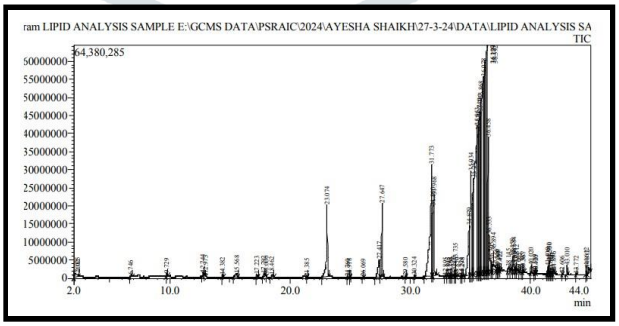

Figure 4: Gas Chromatogram of the lipid extracted from the isolate IS001

Table 3: Components corresponding to the peaks obtained in the gas chromatogram of lipid extracted from the isolate IS001

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Name
1	2.025	1657444	0.03	1588521	Imidazole-5-carboxylic acid, 2-amino-
2	2.081	15327282	0.30	1105098	2-Aminononadecane
3	6.746	3551423	0.07	491751	Octanal
4	9.729	4375060	0.09	923521	Nonanal
5	12.744	2673179	0.05	797615	Decanal
6	12.973	2048122	0.04	712915	Octanoic acid
7	14.382	1927271	0.04	564463	2-Decenal, (E)-
8	15.568	9993868	0.20	981073	Nonanoic acid
9	17.223	2742836	0.05	815314	2-Undecenal
10	17.792	3346958	0.07	693333	Decanoic acid, ethyl ester
11	18.000	768077	0.02	405687	n-Decanoic acid
12	18.462	6288084	0.12	665744	Propane, 2,2-bis(methylthio)-
13	21.385	292437	0.01	118537	Dodecanoic acid, methyl ester
14	23.074	121249015	2.39	19904804	Decanoic acid, ethyl ester
15	24.760	455214	0.01	136090	1,13-Tetradecadiene
16	24.918	587880	0.01	205931	9-Octadecene, (E)-
17	26.069	340980	0.01	133416	Methyl tetradecanoate
18	27.417	47305744	0.93	4874084	Tetradecanoic acid
19	27.647	79641513	1.57	20422082	Tetradecanoic acid, ethyl ester
20	29.580	993585	0.02	361478	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester
21	30.324	1722432	0.03	613857	Hexadecanoic acid, methyl ester
22	31.773	353680781	6.96	30899865	Hexadecanoic acid, ethyl ester
23	31.860	71000265	1.40	19358239	n-Hexadecanoic acid
24	31.968	140403163	2.76	22289639	n-Hexadecanoic acid
25	32.895	669355	0.01	179357	Oleic Acid
26	33.105	458320	0.01	155268	9-Octadecenal, (Z)-
27	33.268	1008899	0.02	277702	Heptadecanoic acid
28	33.354	1185008	0.02	215431	9-Eicosene, (E)-
29	33.603	2490679	0.05	649150	9,12-Octadecadienoic acid, methyl ester
30	33.735	12541972	0.25	4231902	11-Octadecenoic acid, methyl ester
31	33.850	404380	0.01	92910	15-Octadecenoic acid, methyl ester
32	34.224	569410	0.01	210978	Methyl stearate
33	34.298	417293	0.01	150518	Cyclononasiloxane, octadecamethyl-
34	34.879	116443288	2.29	13816818	Linoleic acid ethyl ester
35	35.034	212643986	4.19	28844237	Ethyl Oleate
36	35.257	271841120	5.35	26722737	9-Octadecenoic acid, (E)-
37	35.553	620337859	12.21	41113429	Octadecanoic acid, ethyl ester
38	35.630	140484968	2.77	39663218	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)
39	35.740	275974675	5.43	43441981	6-Octadecenoic acid, (Z)-
40	35.787	146170652	2.88	44791854	Octadec-9-enoic acid
41	35.868	211242087	4.16	48295630	Oxacyclohexadecan-2-one
42	36.078	69491753	13.68	54396378	2,3-Dihydroxypropyl elaidate
43	36.197	358507801	7.06	58629116	Octadec-9-enoic acid
44	36.229	105830985	2.08	59025451	Oleic Acid
45	36.392	648845237	12.77	62858111	Octadec-9-enoic acid
46	36.458	96743620	1.90	37534301	Octadecanoic acid
47	36.533	58070731	1.14	10448326	9,12-Octadecadienoic acid (Z,Z)-
48	36.705	20738868	0.41	3205060	9,12-Octadecadienoic acid (Z,Z)-
49	36.894	35810653	0.70	5751294	9,12-Octadecadienoic acid (Z,Z)-
50	37.156	6768664	0.13	1145985	1-Methylbutyl hexadecanoate
51	37.268	4124612	0.08	825182	Octacosanol
52	37.357	1607737	0.03	437775	Octadecane
53	37.422	1415686	0.03	426977	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester
54	38.145	11863144	0.23	1318939	9,12-Octadecadienoic acid (Z,Z)-
55	38.458	2268944	0.45	4059895	cis-11-Eicosenoic acid
56	38.584	21125057	0.42	4482642	17-Pentatriacontene
57	38.699	5006047	0.10	1368827	n-Tetracosanol-1
58	38.812	9987579	0.20	2410114	Eicosanoic acid
59	39.005	665433	0.01	272473	Heptadecanoic acid, ethyl ester
60	39.305	1565578	0.03	470627	Cyclohexane, 1,1'-dodecylidenebis[4-methyl-
61	39.367	2820330	0.06	717341	Pseduosarsasapogenin-5,20-dien methyl ether
62	40.020	10879563	0.21	2180774	9-Octadecenoic acid, (E)-
63	40.349	1079406	0.02	222457	Hexadecanoic acid, 2-methylpropyl ester
64	40.420	193481	0.00	87245	Heptanoic acid, docosyl ester
65	41.408	9303879	0.18	2268344	Z-13-Octadecen-1-yl acetate
66	41.501	11396748	0.22	3572012	Bis(2-ethylhexyl) phthalate
67	41.550	9667502	0.19	3731060	9-Octadecen-1-ol, (E)-
68	41.626	2520440	0.05	673487	Oxacyclohexadecan-2-one
69	41.797	4088127	0.08	893497	1-Heptacosanol
70	41.896	8133383	0.16	1231265	Octacosyl heptafluorobutyrate
71	42.606	618635	0.01	243277	9-octadecenoic acid, 2,2,2-trifluoroethyl ester
72	43.010	7493885	0.15	2497907	trans-13-Octadecenoic acid
73	43.772	679922	0.01	228296	Cyclononasiloxane, octadecamethyl-
74	44.612	8772271	0.17	2491604	9-Octadecenoic acid (Z)-, octadecyl ester
75	44.812	9540587	0.19	1450786	Octanoic acid, 3-tridecyl ester
		080721852	100.00	753473002	

Conclusion:

The screening of oleaginous yeast from diverse environmental samples yielded promising results. The characterization of crude glycerol obtained from a biodiesel plant indicated its potential as a suitable carbon source. Out of the 35 yeast isolates tested, 29 demonstrated lipid accumulation potential, indicating their suitability for lipid production. Further analysis revealed that the oleaginous yeast isolate IS001 isolated from seawater dockyard exhibited the highest lipid accumulation when grown using crude glycerol and corn steep liquor (CSL) as carbon and nitrogen sources, respectively followed by IS006 and IS007 isolated from fruit waste, IS0015 isolated from used oil from a Mumbai restaurant). Since the lipid content was highest in the isolate IS001 its fatty acid composition was analyzed using GC-MS. The identified fatty acid composition of the lipid extracted from IS001, with the presence of linoleic acid (C18:2), oleic acid (C18:1), and palmitic acid (C16:0), suggests its viability as a feedstock for second-generation biodiesel production, aligning with the optimal fatty acid profile recommended for biodiesel production. Overall, these findings underscore the potential of oleaginous yeast, particularly isolate IS001, in contributing to sustainable biofuel production by utilizing waste resources such as crude glycerol. Further research and optimization of cultivation conditions could enhance lipid productivity and facilitate the commercialization of biofuels derived from oleaginous yeast.

II. Acknowledgment

The authors would like to thank Ruia College (Mumbai) for their invaluable contribution in facilitating the analysis using GCMS.

References

1. Bardhan, P., Gupta, K., Kishor, S., Chattopadhyay, P., Chaliha, C., & Kalita, E. et al. 2020. Oleaginous yeasts isolated from traditional fermented foods and beverages of Manipur and Mizoram, India, as a potent source of microbial lipids for biodiesel production. Annals Of Microbiology, 70(1).
2. Abeln F, Chuck CJ. The history, state of the art, and prospects for Oleaginous Yeast Research. Microbial Cell Factories. 2021 Dec 7;20(1). doi:10.1186/s12934-021-01712-1.
3. Shaikh A, Surti A. Oleaginous Yeast: A Promising Pathway for Biodiesel Production. Journal of Emerging Technologies and Innovative Research. 2024 Feb; 11(2): 2349-5162.
4. Amornrattanapa P, Thongthep P. Isolation and screening of oleaginous yeasts capable of using glycerol as a carbon source. Ramkhamhaeng Research Journal of Sciences and Technology. 2019 Dec 27;22(2):61-70.
5. Awad D, Bohnen F, Mehlmer N, Brueck T. Multi-factorial-guided media optimization for enhanced biomass and lipid formation by the oleaginous yeast *Cutaneotrichosporon oleaginosus*. Frontiers in bioengineering and biotechnology. 2019 Mar 26; 7:54.
6. Chmielarz, M., Blomqvist, J., Sampels, S., Sandgren, M. and Passoth, V., 2021. Microbial lipid production from crude glycerol and hemicellulosic hydrolysate with oleaginous yeasts. *Biotechnology for Biofuels*, *14*, pp.1-11.
7. Bandhu S, Bansal N, Dasgupta D, Junghare V, Sidana A, Kalyan G, Hazra S, Ghosh D. Overproduction of single cell oil from xylose rich sugarcane bagasse hydrolysate by an engineered oleaginous yeast *Rhodotorula mucilaginosa* IIPL32. Fuel. 2019 Oct 15; 254:115653.

8. Caporusso A, Capece A, De Bari I. Oleaginous yeasts as cell factories for the sustainable production of microbial lipids by the valorization of agri-food wastes. Fermentation. 2021 Apr 3;7(2):50.
9. Unver H, Polat E, Altınbaş M. Screening the lipid production potential of oleaginous yeast *Yarrowia lipolytica* under Wood Hydrolysates. Separations. 2023 June 24;10(7):371. doi:10.3390/separations10070371.
10. Shaigani P, Awad D, Redai V, Fuchs M, Haack M, Mehlmer N, Brueck T. Oleaginous yeasts-substrate preference and lipid productivity: a view on the performance of microbial lipid producers. Microbial Cell Factories. 2021 Dec; 20:1-8.
11. Mirza S, Siddique S, Qamer HM, Doggar MG. Optimization of lipid production potential of oleaginous yeast by response surface methodology cultivated in agro-industrial waste. International journal of environmental science and technology. 2019 Jul 1;16:3221-30.
12. Liu LP, Hu Y, Lou WY, Li N, Wu H, Zong MH. Use of crude glycerol as sole carbon source for microbial lipid production by oleaginous yeasts. Applied biochemistry and biotechnology. 2017 Jun; 182:495-510.
13. Shaigani P, Awad D, Redai V, Fuchs M, Haack M, Mehlmer N, Brueck T. Oleaginous yeasts-substrate preference and lipid productivity: a view on the performance of microbial lipid producers. Microbial Cell Factories. 2021 Dec; 20:1-8.
14. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology. 1959 Aug 1;37(8):911-7.
15. Xu J, Zhao X, Du W, Liu D. Bioconversion of glycerol into lipids by *Rhodosporidium toruloides* in a two-stage process and characterization of lipid properties. Engineering in Life Sciences. 2017 Mar;17(3):303-13.
16. Rodríguez-Fernández J, Hernández JJ, Calle-Asensio A, Ramos Á, Barba J. Selection of blends of diesel fuel and advanced biofuels based on their physical and thermochemical properties. Energies. 2019 May 28;12(11):2034.
17. Hu S, Luo X, Wan C, Li Y. Characterization of crude glycerol from biodiesel plants. Journal of agricultural and food chemistry. 2012 Jun 13;60(23):5915-21.
18. Knothe G. Improving biodiesel fuel properties by modifying fatty ester composition. Energy & Environmental Science. 2009;2(7):759-66.
19. Kitcha S, Cheirsilp B. Screening of oleaginous yeasts and optimization for lipid production using crude glycerol as a carbon source. Energy Procedia. 2011 Jan 1; 9:274-82.
20. Chilakamarry, C., Sakinah, A., Zularisam, A. and Pandey, A., 2021. Glycerol waste to value added products and its potential applications. Systems Microbiology and Biomanufacturing, 1(4), pp.378-396.