RESEARCH ARTICLE

Scale up studies on select bacterial strains for improvement of PUFA production

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ABSTRACT
The sustainable production of polyunsaturated fatty acids (PUFAs) from microorganisms has been a focus for several decades. A rising demand for healthy superfoods like PUFA and a decline in their natural sources have prompted the development of faster, more reliable, and more economical microbial production strategies. Unlike algae and fungi, bacteria pose a clear advantage w.r.t. ease of culturing, manipulation, and production speeds. In the present study, two bacterial cultures isolated from the Arabian Sea and one river water *Bacillus* species were studied for their PUFA-producing capability at the lab-scale bioreactor level. These isolates, previously studied for their maximum PUFA output at the shake flask level, were put under lab-scale bioreactor conditions in an attempt to increase productivity. This investigation was carried out using fed-batch and single-batch culture techniques, with regulated environmental parameters, improved aeration, mixing, and a set C: N ratio. With careful monitoring, up to 20% PUFA and 7.9 g/L dry biomass could be obtained from saltwater *Halomonas* spp. While the freshwater *Bacillus thuringiensis* spp. reached a maximum of 11.79% PUFA and 5.6 g/L of dry biomass. The fed-batch mode proved to be more effective, increasing biomass and PUFA production by three to fivefold. Compared to earlier research using the same bacterial isolates in shake flask cultures, the results achieved were significantly higher. Elementary fermentation process data obtained through this investigation will be valuable in promoting further scale-up studies for bacterial PUFA production.

Key words: PUFA, Scale up, Lab-scale bioprocess, *Halomonas*, *Bacillus*, Gas chromatography

Introduction

Polyunsaturated fatty acids (PUFAs) hold an important role in human growth and development. PUFAs like ALA, EPA, and DHA demonstrate extensive benefits for cardiac, reproductive, and nervous system health, including vision and cell signalling systems (Jovanovic et al., 2021; Sun et al., 2022). Humans and mammals cannot synthesise enough PUFA in their bodies; thus, they need to obtain them from dietary sources such as fish and plants (Masurkar et al., 2014). Growing food insecurity and environmental pollutants in fish and plant oils have sparked interest in using more sustainable and affordable alternative resources to produce polyunsaturated fatty acids. Marine bacteria, particularly those belonging to the genera *Shewanella, Photobacterium, Colwellia, Moritella, Psychromonas, Vibrio, and Alteromonas*, are found to be among the major microbial producers of polyunsaturated fatty acids (Moi et al., 2018).

During our previous research, we isolated and identified PUFA-producing bacteria from mesophilic habitats, such as the Arabian Sea and freshwater sources in India, as an attempt to investigate additional bacterial sources of PUFA (Masurkar et al., 2014). The isolates obtained were optimised for PUFA production using shake flask cultivation (Masurkar & Vakil, 2015). Shake flasks can be used fairly successfully for medium development and improving strains. However, for the other aspects of process development, shake flasks are not satisfactory because of factors such as a lack of pH control and the inability to feed the flask without stopping the shaker (Ganjave et al., 2022). Once a culture is confirmed in a shake flask screen for its ability to produce a desired metabolite, it has to be scaled up to be of any commercial benefit. Optimising the fermentation process using a scale-up strategy may often result in a significantly greater improvement than was initially shown in the shaking flask screen (Peñalvaet al., 1998; Rowlands, 2011; Xia et al., 2021)

Scale-up means increasing the scale of fermentation from lab scale to pilot scale to production scale in terms of maintaining volumetric productivity (Stanbury et al, 2013). Scale-up studies help the fermentation technologists to

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increase the scale of fermentation without compromising the product yield obtained on a lower scale. Since small-scale bioreactors more closely mimic industrial bioreactors than shake flasks, they also help in identifying the technical issues that are likely to arise on a real commercial scale (Du et al., 2022; Pretzner et al., 2021).

The facets studied in fermentation processes are the design of optimum medium and inoculum, methods of aeration and mixing, temperature, defoamers, pH control, etc. Modern automation software enables regulation of the fermentation process and captures data in real time. The most promising cultures at the shake flask level have, in some cases, failed at the bioreactor level, according to the literature. So, it becomes imperative to check the reproducibility of the shake flask results at the pilot scale before trying the culture at the industrial level (Rowlands, 2011).

The stirred tank bioreactor (STR) was chosen for the lab-scale bacterial bioprocess studies. STR is the most common type of bioreactor for large-scale bacterial and fungal fermentations due to its *in situ* sterilisation capability, relatively high oxygen transfer rate (OTR) at lower shear rate, environment control, and well-established scale-up relationships (Atkinson & Mavituna, 1991; McNeil & Harvey, 1990).

A number of physical and chemical parameters known as scaling variables influence the behaviour of cultured organisms as the scale of the process becomes larger. The scalable parameters most commonly used in the fermentation industry for efficient scale-up of processes are constant volumetric mass transfer coefficient (KLa), constant impeller tip speed, constant dissolved oxygen concentration and constant specific power input (P/V) (Garcia-Ochoa & Gomez, 2009; Seidel et al., 2023).

Table 1 provides a summary of the process variables that influence mass transfer and mixing.

Table 1. *Process Variables Governing Mass Transfer and Mixing Dynamics (Stanbury et al., 2013).*

Sr. No.	Process variables and units	Mixing characteristics affected
1	Power consumption per unit volume (K/W/hr)	Oxygen transfer rate (OTR)
2	Volumetric air flow rate (VVM)	Oxygen transfer rate (OTR)
3	Height /Diameter ratio (H/D)	Oxygen transfer rate (OTR)
4	Impeller tip speed (m/sec)	Shear

After careful consideration of results from previous studies (Masurkar & Vakil, 2015; Masurkar et al., 2015), 3 high-performing PUFA producers were shortlisted for scale-up studies. The isolates chosen were *Bacillus thuringiensis* spp. from a river in northern India and *Halomonas* spp. and *Bacillus* spp. from the Arabian Sea (Masurkar et al., 2014).

In the current study, select 3 cultures were studied for their single-cell oil and PUFA production efficiency at the lab-scale bioreactor using scale-up strategies like batch and fed-batch processes (Abd Elrazak et al., 2013).

Industrial processes often use a hybrid of the two techniques called fed-batch culture. This involves ordinary batch growth until the carbon substrate is substantially utilised, followed by regular feeding of fresh substrate, which is then rapidly metabolised (Fan et al., 2017). The fed-batch system is a powerful method for overcoming the catabolite repression, which generally decreases the yield of the batch. In case of catabolite repression, bacteria preferably metabolise a particular carbon source first, by inhibiting the synthesis of enzymes involved in catabolism of other carbon sources (Deutscher, 2008). In fed-batch, glucose concentration in the culture liquid is kept low initially, restricting growth and enzyme biosynthesis. It has greater control over the growth and production phase, so final product concentration is greater than achieved in a batch fermentation system, while difficulties associated with obtaining the steady state are minimised (McNeil & Harvey, 1990; Hung et al., 2025).

Scale-up studies were planned on the basis of the results obtained in the shake flask to determine the optimum physical and physiological parameters for PUFA production. It was found that in shake flasks, the cultures produced higher amounts of lipid and PUFA when incubation temperatures and mixing speeds were lowered. As compared to the original medium, freshwater cultures produced more PUFA at pH extremes of 4.0 and 9.0, but saltwater cultures produced the most PUFA at the medium's initial pH. In addition, a C/N=40 gave the best results w.r.t. PUFA productivity without compromising the lipid and biomass production (Masurkar & Vakil, 2015). According to Kennedy et al. (1994a), the ideal medium for shaking flask culture may also be used effectively in a stirred tank. At the same time, it is often observed that quantities of media components used for shake flask processes are not adequate for biomass and/or metabolite production in the bioreactor (Stanbury et al., 2013). To compensate for the same, a fortified medium was used to provide for the highly enhanced conditions of aeration and mixing and pH control that are available in the bioreactor as compared to shake flask conditions.

Materials and Methods

The chemicals and reagents used for work reported in this paper were procured from Merck India Ltd. and from SRL Chemicals, India. Media components were obtained from HiMedia, India.

Culture and growth conditions

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1. Culture No. GN/PA/SL1 (identified as *Bacillus* spp. FPZSP13)*

2. Culture No. GN/PA/H8 (identified as *Halomonas* spp. QY113)*

3. Culture No. GN/PA/N4 (identified as *Bacillus* thuringiensis Bt407)*

(*Masurkar et al., 2014; Masurkar et al., 2015)

Inoculum Preparation

Bacterial inoculum was prepared by harvesting an actively growing culture (18-20 h) from a semi-synthetic MI medium agar slant using 3 cm^3 sterile saline (Masurkar & Vakil, 2015). Optical density of the culture suspension was measured at 530 nm and adjusted to 1.0 O.D. by appropriate dilution. This cell suspension was used as the pre-seed for inoculating the seed flasks. The shake flask inoculum parameters are listed in Table 2.

Table 2. *Parameters for Developing the Seed Culture for Bioreactor Operations.*

Sr. No.	Fermentation Parameters	Shake flask fermentation conditions
1	Seed Medium	Semi-synthetic MI Medium with C/N=40:1
2	Fermentation Volume	4×100cm^3 in 500cm^3 Erlynmeyer flasks
3	Incubation conditions	20^{0}C – 100 rpm
4	Inoculum	5% v/v (5cm^3 of O.D$_{530nm}$=1.0 culture suspension)
5	Duration of incubation	16- 18hr for GN/PA/SL1 while 6-8 h for GN/PA/H8 and GN/PA/N4
6	O.D.	0.8-1.00 A$_{530nm}$ for GN/PA/SL1 while 0.4-0.6 A$_{530nm}$ for GN/PA/H8 and GN/PA/N4
7	Quality Check	Gram staining before inoculation

Inoculum/seed for each of the 3 cultures for inoculation of the bioreactor was prepared in seed medium (composition given in Table 3). The cultures were grown till they reached their early log phase (10-12 h). The O.D. was recorded, and a purity check was performed by Gram staining prior to inoculation.

A 10% v/v (400 cm^3) of seed culture in log phase was used as inoculum for 4 L of culture medium. The O.D. of the culture medium was measured immediately after inoculation as the 'zero' h reading. Thereafter, O.D. measurements were made at regular time intervals.

Preparation of bioprocess medium

To meet the demands of the superior fermentation conditions (temperature regulation, aeration, mixing, and controlled pH) in the lab-scale bioreactor, a fortified semi-synthetic minimal medium (higher glucose and yeast extract concentration) maintaining the C/N ratio of 1:40 was used. While a semi-synthetic medium (C/N=1:40) was used for shake flask experiments, it was also used as a seed medium for developing inoculum.

Table 3. *Composition of seed medium and process medium.*

Medium Components	Seed medium C/N=1:40	Bioreactor medium (Fortified) C/N=1:40
Yeast Extract	2.25g	3.38g
NH_4NO_3	0.43g	0.645g
KH_2PO_4	0.75g	0.75g
$MgSO_4.7H_2O$	0.4g	0.4g
$CaCl_2.2H_2O$	0.4g	0.4g
Glucose (Anhydrous)	40.0g	60.0g
Water	For a total volume of 1000cm3 –> 50% Sea Water 50% Distilled Water for marine cultures, i.e. GN/PA/SL1 and GN/PA/H8, 100% Distilled Water for freshwater culture GN/PA/N4	
pH	5.8 for seawater containing medium & pH adjusted to 9.00 for Distilled water medium using 1N NaOH	
Sterilised by autoclaving at 121ºC for 15 minutes.		

Four litres (4 L) of fortified semi-synthetic minimal medium was prepared for lab-scale fermentation. All the ingredients of the fermentation medium given in Table 3 (except glucose) in required amounts were dissolved in 500 cm^3 of distilled water and transferred into the bioreactor. The calculated amount of glucose was dissolved in distilled water to make up a volume of 1.5 L, which was transferred into the bioreactor to make approximately 2 L of medium.

Then, 2 L of seawater was added to the bioreactor to make up a total volume of 4 L. This medium was used for marine cultures, namely Culture No. GN/PA/SL1 and GN/PA/H8. For the freshwater Culture No. GN/PA/N4, 2 L of distilled water was added to make a total volume of 4 L. Similarly, the seed medium for growing the inoculum to be inoculated into the bioreactor was prepared as per the composition given in Table 3. The calculations for determining the concentrations of glucose and yeast extract for the medium were based on the percentage of carbon in glucose and the percentage of nitrogen in yeast extract (Merck Index, 2009).

Fermentation conditions

Cultivation was carried out in a 6.6 L (maximum working volume of 5 L) stirred tank bioreactor B-Lite, a laboratory bioreactor from Sartorius, Germany, with an H/D ratio of 2.2:1, equipped with a pH electrode [glass electrode (405-DPAS-SC-K8S/320)] and a dissolved oxygen (DO) probe [polarographic probe (Inpro6800/12/320)].

The culture vessel was sterilised in an autoclave (PSM vertical autoclave, 98 L capacity, Bengaluru) at 121°C (15 psi) for 20 min. Ten per cent inoculum was introduced into 4 L of

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fermentation medium. Agitation was provided by a pair of 6-blade Rushton disc turbine impellers (46 mm diameter, 8 mm shaft). The automatic features of the lab bioreactor were operated using SCADA (Supervisory Control and Data Acquisition), a monitoring and control system equipped with Biostat® software for displaying, collecting, and storing information. To assess the cultivation process, several parameters and process conditions were monitored as discussed in Table 4.

Three runs were carried out for each of the 3 cultures under study. The fermentation conditions employed for all three cultures under study are listed in Table 4.

Table 4. *Operating Parameters for Individual Bioreactor Runs*.

Sr. No.	Operating Parameters	Lab-scale fermentation Conditions
1	Fermentation Volume	4L
2	C/N Ratio	40:1
3	Inoculum size	10% v/v (400cm^3)
4	Temperature	20ºC
5	pH	Controlled at 5.8 for GN/PA/SL1 and GN/PA/H8 and controlled at 9.00 for GN/PA/N4
6	Dissolved Oxygen	100% at the start point, further maintained around 30-40%
7	Aeration rate	0.5-1.0 v/v/m
8	Agitation speed	200-500 rpm.
9	Antifoam	10% Silicone
10	Duration	46-48 h

Fed batch

Estimation of the key substrate glucose was performed during each fermentation batch, and when the glucose level fell to half the initial concentration, i.e., around 30-35 g/L, concentrated glucose stock was fed to achieve about 50 g/L concentration.

Process parameters

Parameters like temperature, pH, % DO saturation, and agitation (in terms of RPM) were monitored online. Samples were aseptically withdrawn from the fermentation vessel for analysis of offline parameters and sterility checks. The sterility of the batch was checked by plating (Saltwater Complete Agar plates for Culture No. GN/PA/SL1 & GN/PA/H8 and Nutrient Agar for Culture No. GN/PA/N4) a loopful of a sample withdrawn every 6 h from a running batch and incubating at 25°C for 48 h. Colonies were assessed microscopically for the typical morphology of the respective cultures under study. Furthermore, growing cells were checked for lipid accumulation by Sudan Black B staining. Offline parameters included estimation of biomass by measuring wet weight & DCW (Masurkar et al., 2014). Additionally, residual glucose concentration by the 3, 5-dinitrosalicylic acid (DNSA) method and lipid estimation by gravimetric and SPV (sulpho-phospho-vanillin reaction) methods (Izard & Limberger, 2003) were recorded offline. Lipid profiling by GC analysis was performed as a confirmatory test for determining types and relative concentration (in terms of %) of fatty acids in the lipid produced by cultures under cultivation (Masurkar et al., 2014).

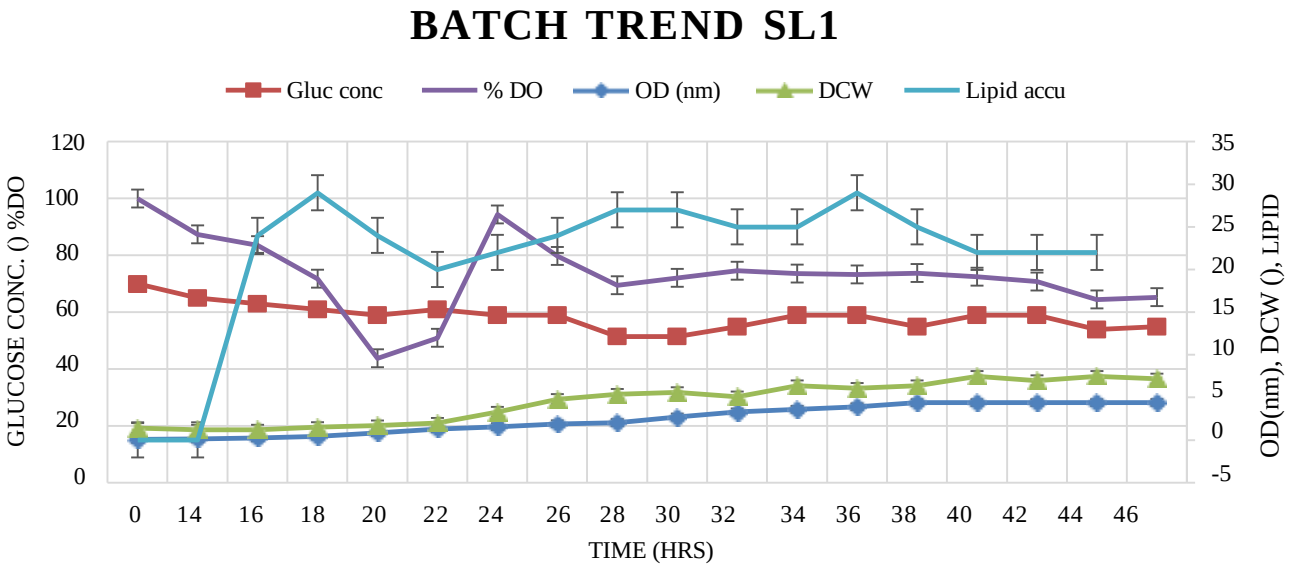

Figure 1. *Key Parameters Monitored During Lab-Scale Fermentation of Culture GN/PA/SL.*

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Fermentation was monitored during the entire batch. Any froth formed was countered by the addition of sterile silicone (30% w/v). Sterilised 1N NaOH or 1N HCl was added as and when required for pH control (pH = 5.8/5.3 ± 0.2).

Harvesting

Fermentation was carried out (for about 48 h) until no appreciable change could be detected in glucose utilisation and lipid concentration (as determined by SPV reaction). Each fermentation batch was harvested in its late stationary phase, when O.D. and dissolved oxygen showed a static trend.

Results

Productivity of saltwater cultures

Isolate No. GN/PA/SL1 (isolated from a saltwater fish, *Sardinia longiceps*): Careful control, regular and frequent monitoring of the fermentation process for Culture No. GN/PA/SL1 resulted in an increased biomass production in the initial growth phase. The corresponding higher demand for nitrogen and carbon was satisfied by the use of a fortified semi-synthetic fermentation medium (Table 2).

Enhanced nutrient supply helped the organism to grow efficiently under the superior culture conditions in the bioreactor. As seen in Figure 1, the Culture No. GN/PA/SL1 reached its stationary phase after 34-36 h of fermentation. The wet weight and DCW estimations for all three batches showed a gradual increase from log phase to stationary phase. Aeration was controlled between 0.75 and 1vvm. A steep drop in DO was observed after 12-18 h of incubation, which was maintained above 40% by adjusting the agitator rpm. There was a small amount of foaming and a slight pH shift into the acidic range, which was brought under control at 5.8 with the addition of 1N NaOH.

The concentration of sugar in the fermented broth decreased with the progress of the fermentation process. The maximum amount of fermentable sugar was consumed by the GN/PA/SL1 cells within 20-36 h. The total lipid in prepared cell suspension samples increased steeply during the log phase and stabilised during the stationary phase (as estimated by the SPV reaction described by Izard & Limberger (2003). It was noted that a fed-batch yielded improved biomass and lipid production up to 22 µg/ml of cell suspension. A single feed of 20 g/L glucose was supplied when the concentration dropped to about half the original concentration. By timely glucose replenishment and oxygen level limitation, a higher percentage of lipid content in biomass and PUFA (7.41%) was achieved in the bioprocess as opposed to the shake flask (Masurkar & Vakil, 2015). This was accomplished after the growth stopped during the second half of the fermentation phase (Table 5). This observation is in agreement with the studies by Kennedy et al. (1994b) on the scale-up of mycelial shake flask fermentations for γ-linolenic acid production by *Mucor hiemalis* IRL 51. Research work by Yamanè & Shimizu (2005) also agrees that fed-batch cultivation helps in overcoming the possibility of enzyme repression caused by excess glucose.

The percentage of total PUFA produced, % MUFA, saturated fatty acids, and other components like short-chain alkanes, sterols, etc., are shown in Table 5. Gas chromatograms showing the profile of the total lipid obtained from three of the GN/PA/SL1 bioprocess batches (RSD < 2%) are shown in Figure 2.

Gas chromatography results suggested that the types and number of PUFA produced differed in the bioprocess

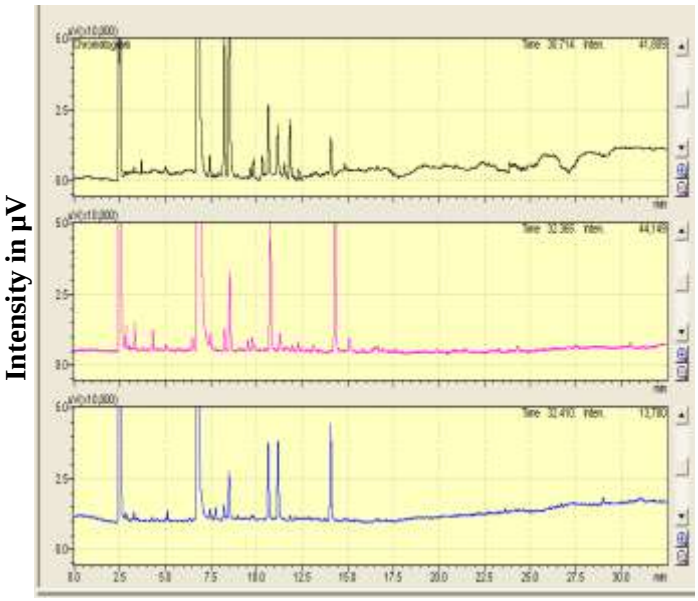

Figure 2. *Lipid Profile of Culture GN/PA/SL1 Cultivated in 5L Bioreactor as Determined by GC Analysis.*

Table 5. *Comparative Estimation of PUFA, MUFA, and Saturated Fatty Acids in Marine and Freshwater Isolates Using GC Analysis.*

Bioreactor Run	PUFA		MUFA		% Saturated Fatty
(Fed-Batch)	Types	%	Types	%	acids and other components
GN/PA/SL1	LA, GLA, EDA, ETA	**7.41**	MA, PA	21.74	70.85
GN/PA/H8	LA, ALA, GLA, EDA, ETA, AA	**20.02**	MA, PA, OA	19.03	60.95
GN/PA/N4	LA, ETA	**5.99**	MA, PA, OA	47.52	53.51

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cultivation as compared to the shake flask culture. Culture No. GN/PA/SL1 produced only linoleic acid in the shake flask, while on cultivation in the bioreactor (which offers enhanced culture conditions like a thermostatic environment, adequate DO, and uniform nutrient accessibility), it produced GLA, ETA, and EDA (Table 5). This culture produced only LA in a shake flask (Masurkar & Vakil, 2015).

Isolate No. GN/PA/H8 (isolated from the Arabian Sea, near the western coast of Maharashtra): Parameters studied during bioprocess runs of Culture No. GN/PA/H8 indicated that the culture entered the log phase around 2-4 h of incubation and reached its stationary phase of growth around 20-22 h of fermentation. Culturing GN/PA/H8 required more diligent control for dissolved oxygen levels and mixing, as DO levels fluctuated frequently after 2-4 h of incubation, following a short lag phase.Aeration was controlled between 0.75-1 vvm using a rotameter to maintain a DO level between 40-60%. Heavy foaming was observed during the log phase, which was controlled by the timely addition of silicone antifoam.

Culture No. GN/PA/H8 attained a high cell density (final O.D. =8.00, DCW=16.8 g/L) at the cost of a high oxygen demand, indicated by the constantly fluctuating DO (Figure 3). The mixing speed was adjusted frequently in the range of 100-500 rpm to maintain the DO around 40%. A drop in the pH was observed from the onset of the log phase till the late log phase, which was controlled at 5.8 by judicious addition of 1N NaOH.

Glucose concentration was seen to fall steadily till 10 h (log phase) of cultivation, accompanied by a steep rise in the total lipid values per ml of cell suspension. A feed of 20 g/L glucose was provided around 34 h, when the glucose dropped to about half the original concentration. The glucose added was utilised to a lesser extent. This may be due to the cessation of the lipogenic phase of the culture, as indicated by a stable lipid accumulation trend in the stationary phase (Figure 3).

Growing Culture No. GN/PA/H8 in a fortified semi-synthetic fermentation medium and cautious control of its fermentation process resulted in an increase in biomass production and percentage lipid content, along with a boost in PUFA production (20.02%) due to controlled pH. Additionally, a controlled pH, sufficient DO availability, and careful process supervision resulted in a boost in PUFA production (20.02%) (Table 5). As compared to its shake flask culture, GN/PA/H8 produced a notable amount of PUFA in the lab-scale bioreactor fed-batch method, ranging from a maximum of 3-8% in the shake flask to as much as 20% in the bioreactor (Masurkar & Vakil, 2015).

This may be due to enhanced oxygen transfer rates provided in the bioreactor. Aeration and agitation provide a well-mixed system, which may be equivalent to the optimum respiration rate required for the organism for better metabolic activity (Shukla et al., 2001). An increase in the aeration rate from 0.2-2.5 vvm enhanced both the specific growth rate and GLA content in experiments conducted on Spirulina platensis by Ronda et al. (2012). In Figure 4, the gas chromatograms showing the total lipid profile from three of the GN/PA/H8 bioprocess batches (RSD < 2%) are presented.

The characteristics of the PUFA generated in bioreactor cultivation differed from shaking flask culture in the case of Culture No. GN/PA/SL1, as was also the case with Culture No. GN/PA/H8 (Masurkar & Vakil, 2015; Masurkar et al., 2015).

While it produced only LA and ETA in a shake flask, upon cultivation in the bioreactor, Culture No. GN/PA/H8 produced

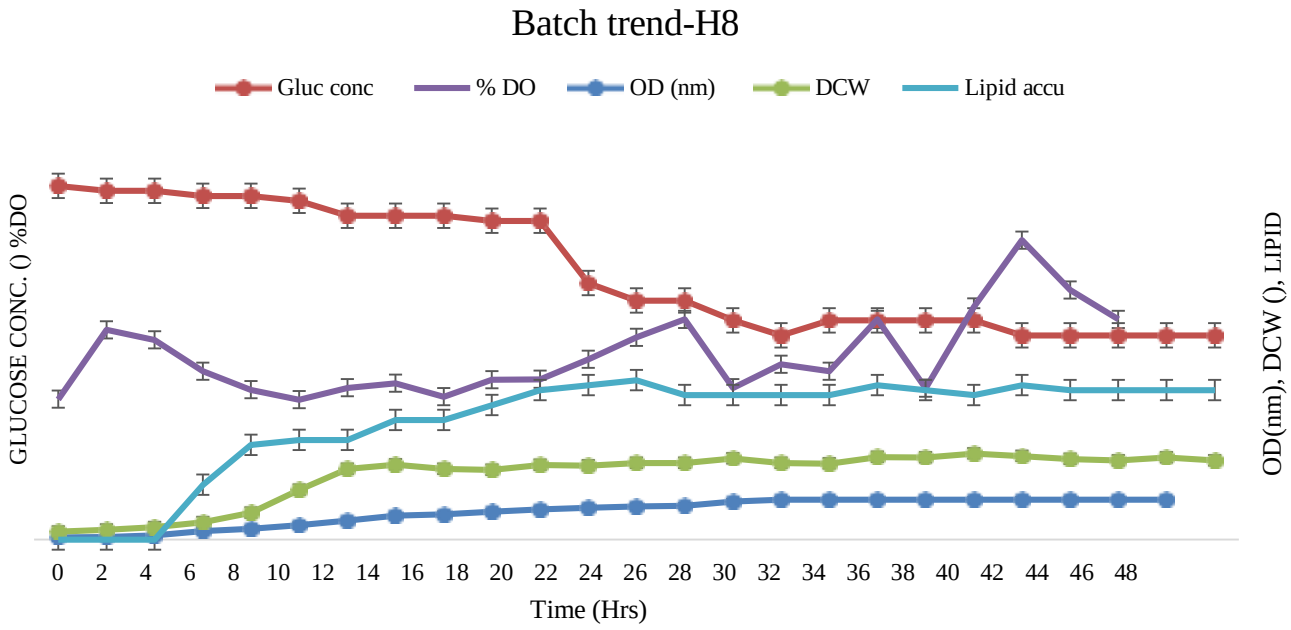

Figure 3. *Online and Offline Parameters Monitored During Three Lab-Scale Fermentation Batches of Culture GN/PA/H8.*

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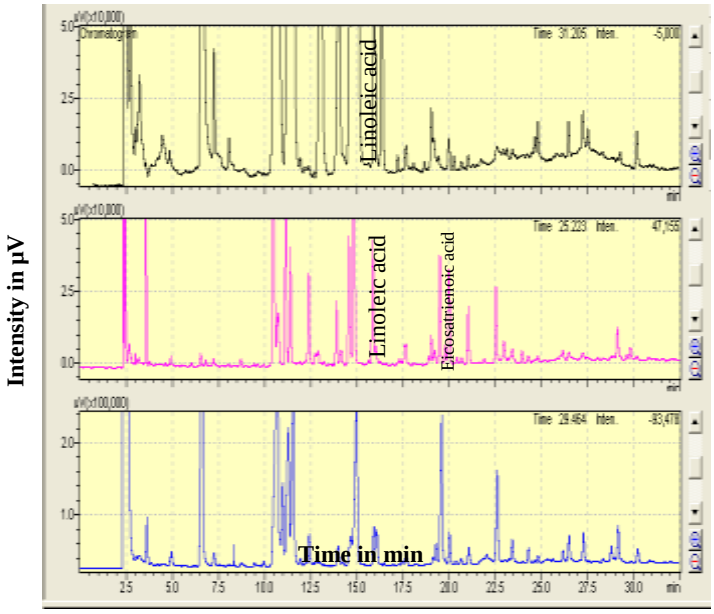

Figure 2. *Lipid Profile of Culture GN/PA/SL1 Cultivated in 5L Bioreactor as Determined by GC Analysis.*

LA, ETA, as well as ALA, GLA, EDA, and AA, which are other PUFAs of commercial interest (Table 5).

Comparing the results for both batch and fed-batch modes of fermentation, it was observed that fed-batch resulted in a better yield (in terms of PUFA) for the second saltwater isolate as well (Table 5, Figures 2 & 4). Research works by Qu et al. (2013) and Chiou et al. (2001) also agree that PUFA production is growth-associated and that fed-batch cultivation helps in overcoming the possibility of enzyme repression caused by excess glucose.

Productivity of freshwater culture

Isolate No. GN/PA/N4 (isolated from the cold-water river, Pindhari, situated in northern India): As shown in Figure 5, the culture GN/PA/N4 reached the stationary phase after 28-34 h of fermentation. Starting with a 6-8 h age seed culture, the biomass gradually increased from log phase to stationary phase, reaching a maximum of 5.6 g/L of dry biomass (Table 5). The DO dropped after 2-4 h of culture, which was then maintained above 40% by adjusting the agitator speed (Figure 5). Little foaming was observed only during the log phase. A deviation in pH from 9.00 to up to 7.1 was observed, which was controlled at 9.00 by the addition of 1N HCl (Masurkar & Vakil, 2015; Abd Elrazak et al., 2013).

The glucose concentrations were seen to drop gradually along the log phase as well as the stationary phase. A glucose feed of 20 g/L was given at 30 h of incubation when the glucose dropped to about half the original concentration. Thereafter, less than half of the glucose feed was utilised by GN/PA/N4 during its 48 h culture duration. The total lipid values per ml of prepared cell suspension sample increased up to 19 µg/cm³ during the log phase, while a drop in the lipid was seen about 26-38 h of incubation until it became steady in the late stationary phase (Figure 5). The culture produced up to 5.99% of PUFA consisting of LA and ETA, while 47.52% was MUFA, mainly MA, PA, and OA, with the rest of the lipid being saturated fatty acids (Table 5). Gas chromatograms showing profiles of the total lipid obtained from 3 bioreactor batches (RSD < 2%) are shown in Figure 6.

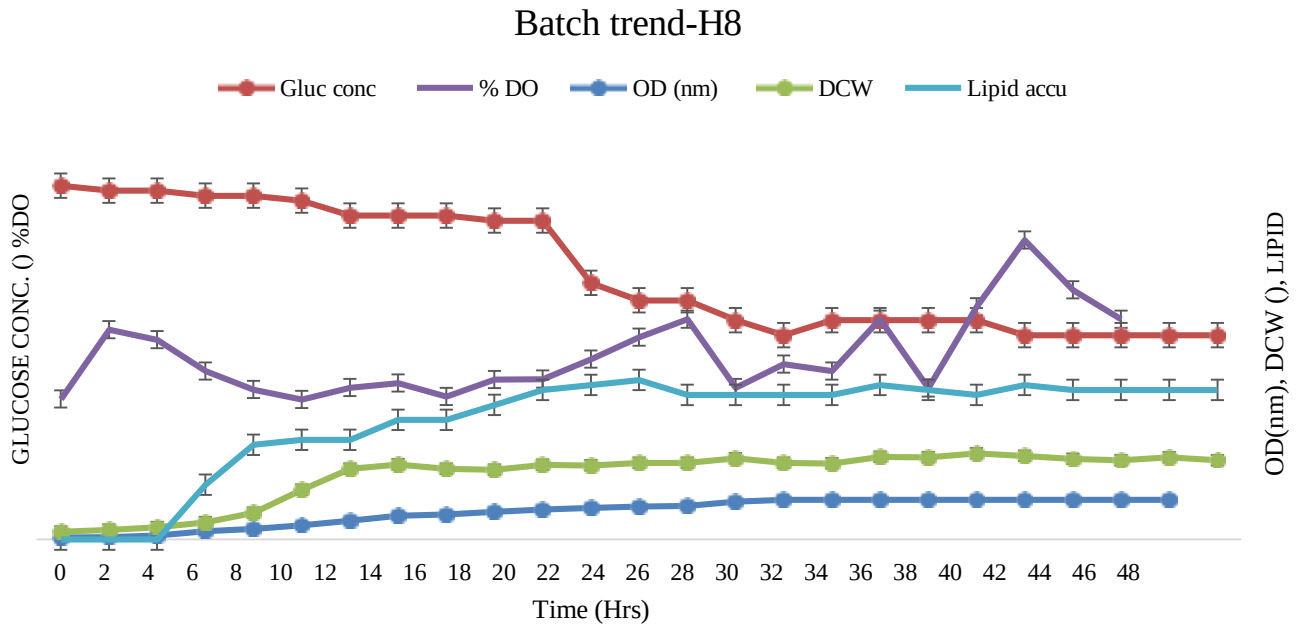

Figure 5. *Process Monitoring of Online and Offline Parameters Across Three Lab-Scale Batches of Culture GN/PA/N4.*

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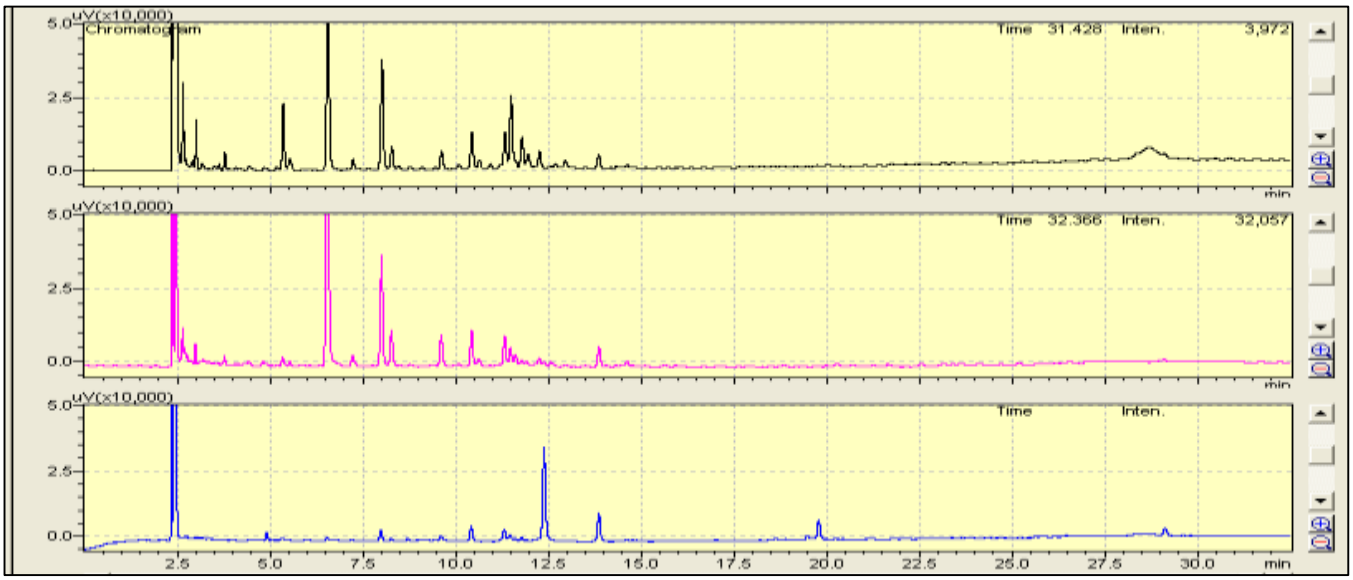

Figure 6. *GC Analysis of Lipid Composition from Culture GN/PA/N4 Grown in 5L Lab-Scale Fermentation.*

Culturing isolate No. GN/PA/N4 in a bioreactor with frequent monitoring of the process resulted in an increased biomass and higher lipid & PUFA production in the fortified semi-synthetic fermentation medium as compared to a shake flask (Masurkar & Vakil, 2015). Culture No. GN/PA/N4 produced only LA in a shake flask, while upon cultivation in the bioreactor with superior culture conditions, it produced ETA (20C-3 unsaturations) in addition to LA (18C-2 unsaturations) (Table 5).

The objective of this study was to determine the total amount of PUFA generated by bacterial cells. To maximise the PUFA production per culture volume, it would be essential to obtain the maximum biomass and intracellular lipid content. Reports suggest that production of PUFA can decrease gradually in prolonged cultivation due to cell lysis (Bajpai & Bajpai, 1992). Organisms use C-source alone for lipid production, growth, metabolism, and maintenance. All these processes also require oxygen. The accumulated lipids are a reserve component that can be consumed when the external C-source is exhausted. Thus, a fed-batch mechanism with close monitoring of glucose levels and their appropriate supplementation can support improving lipid production (Meeuwse, 2011). Whatever the case may be, the challenge is to devise a culture strategy whereby a high cell concentration may be reached without sacrificing a high intracellular oil and PUFA content (Immelman et al., 1997). It is evident from our study that the bioreactor provided superior and controlled culture conditions with minimum temperature fluctuation, sufficient DO, and a homogenous nutrient-rich environment, which facilitated improving the overall growth, lipid, and PUFA production.

Conclusion

The present study explores the parameters for harnessing bacterial potential towards PUFA production in optimal cultivation conditions in a lab-scale bioreactor. Three cultures, namely Culture No. GN/PA/SL1, GN/PA/H8, and GN/PA/N4 were shortlisted for scale-up studies based on their performance on shake flask fermentation. Combining the findings of optimisation studies, the lab-scale bioreactor's PUFA yield increased as a result of improved temperature, pH, mixing, and aeration. Improved lipid and PUFA production was observed in all three cultures under study. Remarkable results were observed for the saltwater isolate No. GN/PA/H8 using the fed-batch strategy, with up to 4.8 g/L of total lipid, of which 20% was PUFA. A considerable variety of PUFAs were also observed, i.e., ALA, GLA, EDA, and AA, in addition to the normally produced LA and ETA. The possibility of using an isolate living in a freshwater source was also evaluated in this study. Comparing results with isolates from saltwater, the freshwater isolate No. GN/PA/N4 could produce a competitive PUFA concentration but less variety. The Ability to obtain PUFA from non-saltwater requiring isolates brings opportunities to reduce the cost of sea salts (or procuring seawater) in formulating the medium. Also, it can eliminate the risk of sea salt residue deposition on the fragile parts of the bioreactor machinery.

Fermentation is a very complex process, and it is often very difficult to obtain a complete picture of what is actually going on in a particular fermentation. The main focus of fermentation is to enhance the production of the desired product, whether it is a primary metabolite, a secondary metabolite,/or biomass. Therefore, optimising the fermentation is critical for ensuring the appropriate growth and product formation. In this

connection, it may be desirable to use a high-density culture as an alternative strategy to enhance PUFA productivity. Among the 3 cultures under study, Culture No. GN/PA/H8 can be considered most suitable for further scale-up studies, considering increased biomass and about a 3-fold increase in PUFA production. No matter what criteria are used for scaling up, it is not possible to reproduce exactly similar conditions on the new scale of operation. Although the results given in this paper are preliminary, they serve as a solid foundation for bacterial PUFA production optimisation and scale-up investigations. There are very few reports on the use of bacterial cultures to produce PUFA. Yeast, fungal, and algal processes are covered in the majority of published material on laboratory, pilot, and commercial-scale PUFA production. Therefore, the outcomes of our laboratory-scale fermentation experiments using bacterial cultures will undoubtedly contribute fresh perspectives to our current knowledge of how to develop an industrial-scale microbial PUFA production process. We believe that bacterial-derived PUFA will eventually compete with conventional sources by enhancing the process, product quality, and lowering costs.

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