Nutritional effects on biomass and metabolic products by *Aurantiochytrium* sp.

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Abstract:

Docosahexaenoic acid, or Docosahexaenoic acid (DHA), plays a pivotal role in biological functions and is beneficial for both humans and animals. Traditionally, DHA is sourced from fish and fish oil. Due to the disadvantages associated with these sources, such as marine pollution and variable composition, there is a pressing need to explore alternative, reliable sources for DHA. *Aurantiochytrium* sp. has been identified as a promising candidate for DHA production. The primary aim of this study was to cultivate three strains in previously published media and, subsequently, to develop defined or semi-defined media for *Aurantiochytrium* sp.. These strains were grown in the selected media, and the dry weight of the cells was measured. Total fatty acids were extracted and analysed using gas chromatography. All three strains demonstrated satisfactory growth in media that incorporated glucose as a carbon source and monosodium glutamate as a nitrogen source. Strain B072 achieved the highest DHA concentration, with a peak of 5.0 g/l, and could accumulate lipids up to 57.57% (w/w). The DHA content in the biomass was 21.43% (w/w), with a biomass yield of 23.35 g/l. Vitamins and trace elements positively influenced the growth and DHA production of *Aurantiochytrium* sp.. Furthermore, strain B072 efficiently utilised ammonium for growth and fatty acid production.

Keywords: *Aurantiochytrium* sp., defined media, DHA production, metabolites, nutrient.

Classification numbers: 3.4, 3.5

1. Introduction

There is little doubt that omega-3 fatty acids hold significant importance in human nutrition. DHA is a primary structural component of the phospholipid membranes of tissues throughout the body, particularly prevalent in the retina, the grey matter of the brain, and the spermatozoa. Notably, in these areas, DHA (C22:6n-3) constitutes up to 36.4% of total fatty acid [1, 2]. As components of cell membranes, omega-3 fatty acids enhance membrane fluidity, which influences a variety of membrane functions, including eicosanoid signalling, ion channel modulation, and regulation of gene expression [3, 4]. Dietary omega-3 fatty acids reduce the risk of cardiovascular disease (CVD). Both epidemiological and interventional studies have shown the beneficial effects of omega-3 fatty acids on various CVD endpoints, encompassing all CVD (defined as all coronary artery disease [CAD], both fatal and nonfatal myocardial infarction, and stroke combined) [5, 6]. DHA is crucial for the growth and functional development of the brain in infants. This fatty acid is transferred across the placenta and accumulates in the brain and other organs during foetal development. Depletion of DHA from the retina and brain results in diminished visual function and learning deficits, which may stem from DHA's essential role in membrane-dependent signalling pathways and neurotransmitter metabolism. DHA is also necessary for maintaining normal brain function in adults. A diet rich in DHA enhances learning ability, while DHA deficiencies correlate with learning deficits. The brain prefers DHA over other fatty acids. DHA turnover in the brain is rapid, more so than often realised. The visual acuity of healthy, full-term, formula-fed infants is enhanced when their formula contains DHA [7]. Over the past 50 years, numerous infants have consumed formula diets devoid of DHA and other omega-3 fatty acids. DHA deficiencies are linked with foetal alcohol syndrome, attention deficit hyperactivity disorder, cystic fibrosis, unipolar depression, and aggressive hostility. A decrease in the concentration of DHA in the brain are associated with cognitive degradation during ageing and with the onset of sporadic Alzheimer's disease [7, 8]. Are there detrimental effects for humans supplementing their diet with DHA? Numerous reports address this issue. Generally, DHA showed no toxicity when supplemented at low concentrations. For instance, oil from *Schizochytrium limacinum* strain FCC-3204, rich in DHA, was proposed

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as a novel food (NF). The applicant recommended its use in infant formulas (IF) and follow-on formulas (FOF) with DHA at levels of 20-50 mg/100 kcal. It was concluded that the NF is safe under the proposed conditions [9]. Docosahexaenoic acid-rich microalgae (DRM) from *Schizochytrium* sp. was given to groups of male and female Sprague-Dawley rats for at least 13 weeks. This study found that DRM supplementation showed no adverse effects in rats relevant to humans at dosages up to 4000 mg/kg/d [10]. The fatty acid profiles of microorganisms are swayed by environmental conditions. The medium's composition is one of the most important environmental criteria in determining final lipid composition. The concentration of carbon and nitrogen in the medium can affect DHA and lipid content, and a high carbon-to-nitrogen ratio often enhances the total lipid content and DHA yields [11].

Carbon and nitrogen sources, along with other cultivation conditions, were explored in batch fermentation to optimise DHA production from strains of the genera *Schizochytrium* and *Thraustochytriidae* [12]. Monosaccharides, glucose, and fructose proved beneficial for cell growth. Glucose emerged as the most effective carbon source for biomass, lipid, and DHA production. Though *Schizochytrium* sp. S31 could utilise disaccharides such as lactose, maltose, sucrose, and soluble starch for cell growth and lipid production, they were suboptimal for DHA production [13]. The highest DHA content (28% w/w) was noted with glycerol as a carbon source, whereas maltose and lactose yielded poor growth [14]. While glucose was generally preferred, some strains favoured fructose. For example, fructose emerged as the most suitable substrate for biomass, lipid, and DHA content in *Aurantiochytrium* sp. SW1 [15]. Both glucose and starch yielded high DHA levels, with 0.41 and 0.65 g/l, respectively, and total fatty acids of 40.8 and 49.0% (w/w) [16]. The effects of different carbon sources were studied in *Thraustochytrium* sp. ATCC 20892, with glucose producing optimal DHA yields even though biomass yields with glucose, starch, and linseed oil were consistent. However, maltose and sucrose as carbon sources resulted in inferior biomass and DHA yields, as observed by A. Singh, et al. (1996) [17]. This study seeks to investigate the influence of trace elements and vitamins, alongside defined MSG media, on the growth and DHA production in the valuable genus *Aurantiochytrium*.

2. Materials and methods

2.1. Materials

Preparation of inoculum: *S. mangrovei* Sk-02 (Sk-02), *S. mangrovei* strain B072, and *S. limacinum* B013 (B013) were employed in this study. These strains are most likely new species in the genus *Aurantiochytrium* [18, 19]. Pure cultures were maintained on slants in solid GPY-medium containing 15 g/l agar and artificial sea salts (ASS, Sigma, USA) at 20°C, and were sub-cultured monthly. To prepare the inoculum, cells from a slant were streaked onto an agar plate and incubated at 25°C for two days. Using a Pasteur pipette, circular 1 cm diameter wells were made in the agar, and the plate was flushed with 10 ml of sterile ASS adjusted to 15%. After 2-3 h, zoospores accumulated in the wells, and this zoospore suspension served as the inoculum. 1 ml of zoospore suspension was transferred into 50 ml of sterile broth medium containing 1 g of Difco yeast extract (DYE), 1 g of peptone (Difco), 10 g of glucose, and 1 l of ASS at 15‰ in a 250 ml Erlenmeyer flask. The flasks were incubated at 25°C and agitated at 200 rpm for 24 h. These cultures were used as inoculums for subsequent cultivation. The broth in the inoculum flasks was harvested and centrifuged at 5,000 rpm for 10 min, then washed with 2-morpholino ethanesulfonic acid (MES). Afterwards, 5 ml of the cells were transferred into 100 ml of the subsequent medium.

Vitamins and trace elements were standardised from various literature sources [20]. A stock solution of a vitamin mixture was prepared, filtered using 0.2 μm filter paper (Minisart-Sartorius), and stored in a refrigerator. The components of the vitamin mixture per litre included: biotin 0.05 mg, para-aminobenzoic acid 0.20 mg, myo-inositol 25 mg, and 1 mg each of calcium pantothenate, nicotinic acid, thiamine HCl (B1), pyridoxine HCl (B6), riboflavin (B2), and cyanocobalamin (B12). The trace elements consisted of: $ZnSO_4 \cdot 7H_2O$ 4.5 mg, $CoCl_2 \cdot 6H_2O$ 0.3 mg, $MnCl_2 \cdot 4H_2O$ 1.0 mg, $CuSO_4 \cdot 5H_2O$ 0.3 mg, $CaCl_2 \cdot 2H_2O$ 4.5 mg, $FeSO_4 \cdot 7H_2O$ 3.0 mg, and $Na_2MoO_4 \cdot 2H_2O$ 0.4 mg per litre. The components of the media are detailed in Tables 1 and 2.

Table 1. Components of defined MSG media M19-19E.

Component (g/l)	M19	M19A	M19B	M19C	M19D	M19E
Glucose	60	60	60	60	60	60
MSG	10	10	10	10	10	10
ASS	15	15	15	15	-	-
K_2HPO_4	0.3	0.3	0.3	0.6	0.3	0.3
NaCl	-	-	-	-	11.98	24
$MgSO_4 \cdot 7H_2O$	-	-	-	-	3.08	12
$CaCl_2$	-	-	-	-	-	1
KCl	-	-	-	-	-	0.75
Vitamins	+	Reduce 5-fold	Reduce 20-fold	+	+	+
Trace elements	+	+	+	+	+	+

Table 2. Components of $(NH_4)_2SO_4$-MSG and MSG (5 g/l) media M20 and M27.

Component (g/l)	M20	M21	M22	M23	M24	M25	M26	M27
Glucose	30	30	30	60	30	30	30	30
DYE	2.5	-	5	-	2.5	-	-	-
$(NH_4)_2SO_4$	1.25	2.5	-	2.5	-	-	1.25	-
K_2HPO_4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
$NaNO_3$	-	-	-	-	1.5	3	-	-
MSG	-	-	-	-	-	-	2.5	5
ASS	15	15	15	15	15	15	15	15
Vitamins	+	+	+	+	+	+	+	+
Trace elements	+	+	+	+	+	+	+	+

2.2. Dry weight determination

3 ml of broth culture was transferred into weighed centrifuge Eppendorf tubes, then centrifuged at 12,000 rpm for 10 min. The supernatant was subsequently separated. Harvested cells were rinsed thrice with distilled water, freeze-dried for 24 h, and weighed. Biomass values are presented as the means of three measurements along with their standard deviation [21]. Supernatants from the biomass were employed for glucose determination using a commercial enzymatic Glucose KIT (Human, Germany).

2.2.1. Fatty acids analysis

Freeze-dried cells (20 mg) were weighed and placed into dark vials. They were then subjected to acid-catalysed transesterification in 2 ml of 4% sulphuric acid in methanol. Heptadecanoic acid (C17-Sigma) acted as the internal standard, with butylated hydroxytoluene (BHT) added as an antioxidant (0.1 g/l). After vortex mixing, the vials were incubated in a water bath at 90°C for 1 h. Post-cooling to room temperature, the mixture was extracted twice with a combined 1 ml of hexane and 1 ml of distilled water. Anhydrous sodium sulphate (Na_2SO_4) grains were added to absorb any residual water. Extracted samples were introduced into the gas chromatograph (GC-17A-Osaka, Japan). Fatty acid methyl esters were differentiated using gas chromatography fitted with a flame ionisation detector (FID) and an Omegawax 250™ 30 m×0.25 mm×0.25 μm film thickness column. The injector was set at 250°C, accepting an injector volume of 1 μl. The column temperature was held at 200°C for first 10 min then increased up to 230°C at a rate of 10°C/min, where it remained for 14 min. Helium (He) served as the carrier gas. Fatty acid methyl esters were identified by chromatographic comparison with authentic standards (DHA-Sigma). The quantities of fatty acid methyl esters were derived from the peak areas in relation to the internal standard C17.

2.2.2. Calculations and definitions

Total fatty acid (TFA) content (% w/w) is calculated as: [Total fatty acid content (g) x 100]/Biomass (g). Percentages of individual fatty acids were determined in both the biomass and lipid. Individual fatty acids in biomass (% w/w) is: [Each fatty acid (g/l) x 100]/Biomass (g/l). Individual fatty acids in lipid (% of TFA) is: [Each fatty acid (g) x 100]/Total fatty acid content (g). TFA (g/l) is: [TFA (%w/w) x Biomass (g/l)]/100. DHA (g/l) is: [DHA (% w/w) x Biomass (g/l)]/100.

Statistical analysis entailed a one-way analysis of variance (ANOVA). If the ANOVA yielded significant results ($p \leq 0.05$), the Student-Keuls-Newman tests were applied to discern pairwise treatment differences.

3. Results

3.1. Growth characteristics and fatty acids formation of three strains in MSG medium M19

Three strains were cultivated in medium M19 (Fig. 1). All strains demonstrated favourable growth in medium M19, where MSG functioned as the sole nitrogen source. The strain Sk-02 peaked in biomass when glucose was fully consumed. In contrast, strain B072 achieved its highest biomass at 22.4 g/l, and B013 reached 19.2 g/l when glucose was nearly depleted.
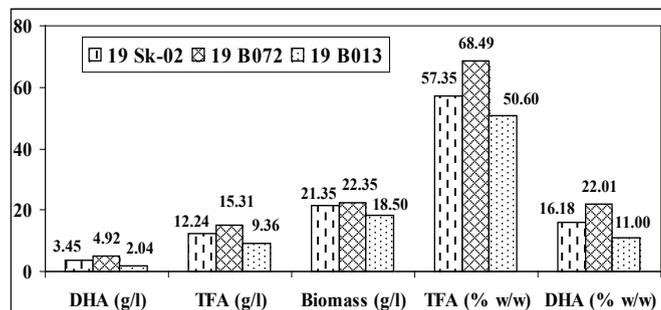

Fig. 1. DHA, DWC, TFA of three strains in medium M19.

B072 was cultivated in media M19A, M19B, and M19C. In medium M19C, strain B072 attained its maximum biomass of 23.35 g/l. In other media, B072 consistently reached its peak biomass when glucose was entirely exhausted (Fig. 2).
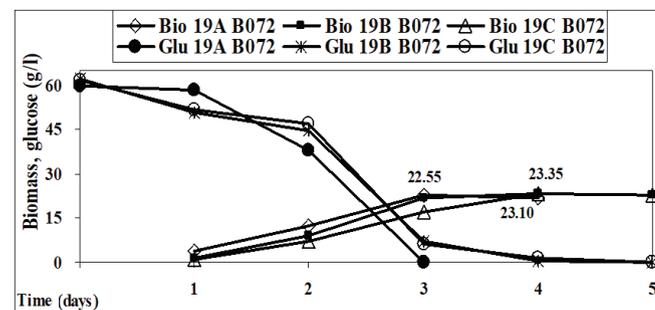

Fig. 2. Biomass and glucose levels for B072 modified MSG media M19A-C.

When cultivated in medium M19A (with vitamins reduced fivefold), B072 reached a maximum biomass and accumulated lipid up to 64.9% (w/w) with a DHA content of 17.74% (w/w). Consequently, DHA yield was 4.0 g/l, and the total fatty acids reached 14.6 g/l. In medium M19B (with vitamins reduced by a factor of 20), the biomass peaked at 23.1 g/l, accumulating lipid up to 47.8% (w/w) with a DHA content of 14.96% (w/w). Thus, DHA amounted to 3.46 g/l and total fatty acids to 11.04 g/l. When compared to medium M19, outcomes from M19A and M19B were inferior. These data clearly demonstrate the influence of vitamins on the growth and fatty acid formation of B072.

B072 was subsequently cultivated in medium M19C (Table 1). This medium differed from M19 by containing 0.6 g/l of K_2HPO_4 instead of 0.3 g/l. Results showed modest increases in both biomass (23.35 g/l) and DHA (5.0 g/l), with no significant difference in DHA level compared to medium M19.

In medium M19, where ASS functioned as the primary sodium source, B072 displayed robust growth (Fig. 3). To devise a cost-effective medium, ASS was replaced with different sea salt components, leading to the creation of media M19D and M19E.
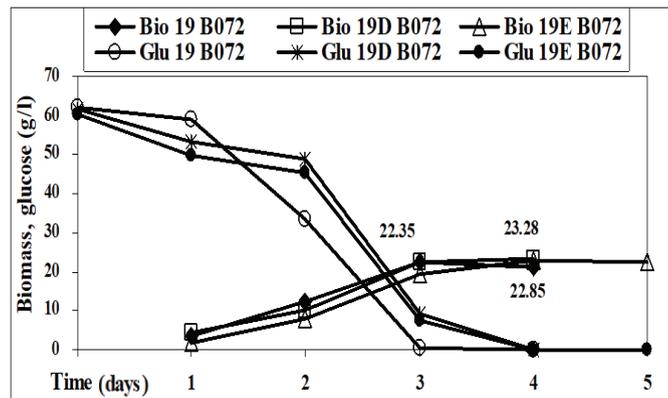

Fig. 3. Biomass and glucose levels for B072 in control medium M19 and media with individual salts replacing ASS (M19D and M19E).

B072 attained maximum biomass levels of 22.35 g/l in M19, 23.28 g/l in M19D, and 22.85 g/l in M19E. In M19, B072 achieved a DHA maximum of 4.9 g/l and a biomass of 22.35 g/l. In M19D, the figures were 4.4 g/l for DHA and 22.5 g/l for biomass, while in M19E, DHA and biomass values were 4.05 and 22.33 g/l, respectively. Results highlighted that while there was a significant ($p \leq 0.05$) reduction in DHA, biomass yield remained unaffected when specific salts replaced ASS. The study's primary focus remained on B072 due to its promising results in prior experiments.

3.2. Growth characteristics and fatty acids formation of B072 in modified MSG media

B072 was cultivated in media M19A, M19B, and M19C. The strain B072 achieved its peak biomass level at 23.35 g/l in medium M19C. Similarly, in other media, B072 consistently reached its peak biomass when glucose was fully consumed.

In medium M19A, where vitamins were reduced fivefold, B072 reached a maximum biomass of 22.6 g/l and accumulated lipids up to 64.89% (w/w). The DHA content in the lipid was 17.74% (w/w), resulting in a DHA yield of 4.0 g/l and a total fatty acid count of 14.6 g/l. When cultivated in medium M19B, where vitamins were reduced by a factor of 20, the biomass peaked at 23.1 g/l. The lipid accumulation was 47.8% (w/w), and the DHA content was 14.96% (w/w). As a result, DHA reached 3.46 g/l and total fatty acids totalled 11.04 g/l. Compared to medium M19, the results from mediums M19A and M19B were consistently lower. It is evident from these results that the vitamin content influences the growth and fatty acid formation in B072.

B072 was subsequently cultivated in medium M19C, which was a modification of medium M19, containing 0.6 g/l of K_2HPO_4 instead of the 0.3 g/l found in M19. The results, as illustrated in Table 3, indicated slight increases in biomass (23.35 g/l) and DHA (5.0 g/l). Nevertheless, the difference in DHA levels compared to medium M19 (the control medium) was not significant.

Table 3. Metabolites of strain B072 in M19-M19E.

Strain B072	M19 Day 3	M19A Day 3	M19B Day 4	M19C Day 4	M19D Day 3	M19E Day 5
Biomass (g/l)	22.35	22.55	23.10	23.35	22.50	22.33
TFA (g/l)	15.31	14.63	11.04	13.44	15.12	13.52
DHA (g/l)	4.92	4.00	3.46	5.00	4.40	4.05
TFA (%w/w)	68.49	64.89	47.80	57.57	67.19	60.55
DHA (%w/w)	22.01	17.74	14.96	21.43	19.56	18.14
FA compositions (% of TFA)						
C12	-	0.51	0.00	0.34	-	0.60
C14	7.81	10.56	8.20	7.23	8.37	11.34
C15	0.67	0.58	0.74	1.00	0.61	0.57
C16	51.78	53.67	51.70	45.21	54.90	50.11
C18	0.98	0.88	0.96	0.78	0.96	0.86
C20:3n3	0.70	0.63	0.66	0.94	0.61	0.79
C20:4n6	-	0.38	0.00	0.44	-	0.41
C20:5n3	-	0.00	0.00	0.44	-	0.47
DPA	5.91	5.45	6.45	6.40	5.45	4.90
DHA	32.14	27.34	31.29	37.22	29.11	29.97

3.3. Growth characteristics and fatty acids formation of B072 in DYE-ammonium sulphate (NH₄)₂SO₄ media M20-M23

Medium M20 comprised both DYE and $(NH_4)_2SO_4$. Medium M21 contained only $(NH_4)_2SO_4$, serving as the sole nitrogen source, while Medium M22 had only DYE. Medium M23 was identical to M21 but had 60 g/l glucose instead of 30 g/l (Fig. 4).
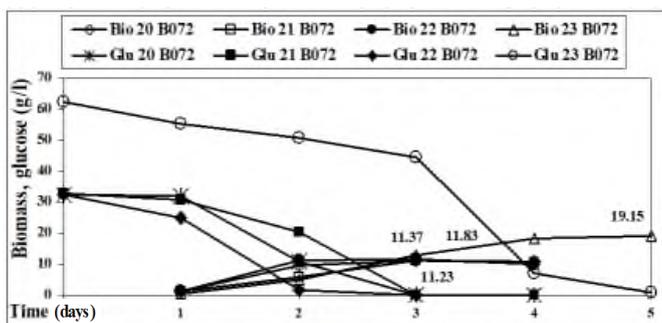

Fig. 4. Glucose and biomass levels of strain B072 in M20-M23 media.

In medium M21, B072 registered the lowest DHA level at a maximum of 0.88 g/l and the lowest lipid content in the biomass, reaching a peak of 42.33% (w/w). The DHA content in the biomass reached merely 8.46% (w/w). In medium M20 of DYE and inorganic nitrogen, B072 achieved a maximum of 1.28 g/l DHA and 11.27 g/l biomass, accumulating up to 53.38% (w/w) in total fatty acids. Medium M22, using DYE exclusively as the primary nitrogen source, yielded superior results compared to medium M21, which lacked any organic nitrogen sources. Strain B072 demonstrated substantial growth in medium M21 with 60 g/l glucose and ammonium sulphate as the exclusive nitrogen source, resulting in peak DHA and biomass levels of 2.73 and 19.15 g/l, respectively (Table 4).

Table 4. Biomass and metabolites of the strain B072 in M20-23.

Media	Strains	Biomass (g/l)	TFA (g/l)	DHA (g/l)	TFA % w/w	DHA % w/w	DHA % of TFA
M20	B072	11.27±0.56	6.07	1.28	53.38	11.27	23.91
M21	B072	11.23±0.61	4.74	0.88	42.33	8.46	19.98
M22	B072	11.83±0.59	6.14	1.65	65.30	16.13	28.00
M23	B072	19.15±1.05	11.10	2.73	60.16	14.78	24.99

3.4. Growth characteristics and fatty acids formation of B072 in DYE-sodium nitrate (NaNO₃) media M24 and M25

The constituents of mediums M22, M24, and M25 are presented in Table 5. While medium M22 exclusively utilised DYE as a nitrogen source, B072 reached peak biomasses of 10.93 and 11.93 g/l in media M24 and M27, respectively. By the third day of cultivation, glucose was entirely consumed.

B072 demonstrated growth in medium M25, where $NaNO_3$ acted as the sole nitrogen source. However, this strain yielded significantly lower biomass and DHA levels. The addition of DYE to medium M24 enabled this strain to grow more effectively, producing a higher DHA quantity than in medium M25, which contained only $NaNO_3$ as a nitrogen source. In medium M24, B072 achieved peak levels of 10.93 g/l biomass and 1.89 g/l DHA.

Table 5. Biomass and metabolites of three strains in M24-M27.

Media	Strains	Biomass (g/l)	TFA (g/l)	DHA (g/l)	TFA %w/w	DHA %w/w	DHA % of TFA
M24	B072	10.93±0.66	6.77	1.89	70.18	17.45	29.46
M25	B072	5.40±0.25	3.68	0.85	68.19	15.67	24.17
M26	B072	12.07±0.66	6.38	1.79	52.86	14.87	28.13
M27	B072	11.93±0.61	7.10	1.44	59.51	13.80	25.75

3.5. Growth characteristics and fatty acids formation of B072 in ammonium sulphate-MSG media M26 and M27

B072 was cultivated in medium M26, which combined the nitrogen sources of MSG and ammonium sulphate $(NH_4)_2SO_4$. B072 achieved its peak biomass level of 12.07 g/l when glucose was fully depleted, accumulating lipids up to 52.86% (w/w). The DHA content in the biomass was 14.78% (w/w). Therefore, this strain reached a maximum), translating to a DHA yield of 1.79 g/l DHA. In medium M27, which used MSG as the sole nitrogen source, B072 reached a biomass peak of 11.93 g/l and accumulated lipids up to 59.51% (w/w) with a DHA content of 13.80% (w/w), yielding 1.44 g/l DHA. These results conclusively demonstrate that B072 can effectively grow and produce DHA in media with inorganic nitrogen sources, such as ammonium.

4. Discussion

In previous experiment, all media were prepared either without yeast extract or with very minimal amounts to minimise the presence of amino acids, vitamins, and trace elements found in DYE. This approach was taken to evaluate the effects of vitamins and trace elements. The results demonstrated significantly low biomass and DHA levels for all three strains. These findings underscored the role of vitamins and trace elements on the growth and DHA production of Sk-02, B072, and B013, especially when comparing the biomass and DHA levels of these strains in pairs of media M12/M13 and M14/M15 [22]. I. Iida, et al. (1996) [23] revealed that vitamins and trace elements positively influenced the growth and DHA production of *T. aureum*. The significance of vitamins and trace elements was also corroborated for *S. mangrovei* Sk-02 [15].

ASS was substituted with sea salt components to reduce costs and allow better control of individual salt levels. Using sea salt components could achieve similar results as ASS, particularly for strain B072. Another study found that DHA weight contents decreased with higher temperatures. The influence of salinity was ambiguous. The peak DHA content (28% w/w) was observed with glycerol; biomass and DHA levels reached their zenith at 25 g/l ASS. Replacing ASS with different sodium salts led to reduced biomass and DHA yields [24].

All three strains were cultivated in a defined MSG medium and its variants. These strains thrived relatively well, producing substantial amounts of DHA in the defined MSG medium M19 with MSG as the lone nitrogen source. The role of vitamins and trace elements became evident as B072 exhibited reduced biomass and DHA in vitamin-deficient media (M19A and M19B). The phosphate effect was assessed in medium M19C, which contained 0.6 instead of 0.3 g/l, as in medium M19. Both biomass and DHA saw minor increments. The replacement of ASS with a streamlined sea salt component formulation yielded equivalent results in biomass and DHA production (M19D, E vs M19). Moreover, medium M19D, containing a reduced amount of NaCl, may be viewed as a cost-effective option for large-scale fermentation.

Lastly, B072 cultivation was explored in media with combined nitrogen sources (ammonium sulphate-DYE, sodium nitrate-DYE, and MSG-DYE media). Table 8 results indicated that the ammonium sulphate-DYE medium resulted in superior DHA levels compared to the sole ammonium sulphate medium. A similar trend was observed in the nitrate-DYE medium. In juxtaposition with results from medium M22, which only contained DYE, it is inferred that B072 can utilise both ammonium and nitrate as nitrogen sources. Nonetheless, nitrate did not emerge as a suitable nitrogen source for B072. The findings in Table 5 illustrated that with nitrate as the primary nitrogen source, this strain assimilated glucose quite slowly, resulting in low biomass levels (M25). The nitrogen utilisation of *S. mangrovei* B072 paralleled that of *T. aureum* [25], *S. limacinum* SR21 [26], and *S. mangrovei* Sk-02 [12]. This study's outcomes signified that monosodium glutamate combined with ammonium sulphate was more efficacious as nitrogen sources for DHA production compared to monosodium glutamate on its own. This aligns with previous reports [27]. Notably, for *T. aureum*, optimal DHA levels were achieved by mixing monosodium glutamate with yeast extract [23]. In a different study, six media with diverse nitrogen sources were examined to pinpoint the most favourable medium for producing high quantities of DHA, biomass, and overall fatty acid content. The peak biomass (17.1 g/l) and DHA

(2 g/l) yields were achieved in a fed-batch system using sodium glutamate, $(NH_4)SO_4$, KH_2PO_4, and $MgSO_4$, after a 12-day incubation in the fed-batch medium of *T. roseum*. Here, the total lipid content stood at 48.8% (w/w) [17]. Similarly, a *Thraustochytrium* sp., closely related to *T. striatum* T91-6, managed to produce up to 28 g/l biomass, 81.7% (w/w) TFA, 31.4% (w/w) biomass DHA, and 4.6 g/l DHA under optimal cultivation condition withconditions; a shaker at 130 rpm and 25°C in a medium comprising 60 g/l glucose, 10 g/l yeast extract, and 2 g/l sea salt [28].

5. Conclusions

Aurantiochytrium sp. thrived and efficiently produced DHA in media sea salt components (sodium chloride, magnesium sulphate, sodium sulphate) and when replacing ASS media. Strain B072 was adept at utilising both $(NH_4)_2SO_4$ and $NaNO_3$ for growth and DHA production. Additionally, this strain harnessed ammonium for growth and fatty acid synthesis. Although strain B072 could grow using $NaNO_3$ as the sole nitrogen source, it resulted in meagre biomass and DHA production. Introducing yeast extract into the medium made strain B072 flourish and produce more DHA compared to a medium with only $NaNO_3$ as the nitrogen source, indicating the inadequacy of $NaNO_3$ as a primary nitrogen source for B072. Glutamate emerged as a potent nitrogen source for a defined medium, especially for B072. These findings suggest that defined media supplemented with vitamins and trace elements could be ideal candidates for *Aurantiochytrium* with an aim to enhance DHA production.

CRediT author statement

Pham Thi Mien: Methodology, Investigation, Writing original draft preparation; Dao Viet Ha: Data curation, Resources, Validation; Cornelis Verduyn: Conceptualisation, Resources, Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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