Effect of Sodium and Potassium Ions on the Growth and Fatty Acid Composition of *Dunaliella salina*

V. Devi* and Vibha Dhawan

Biotechnology and Bioresources Division, The Energy and Resources Institute (TERI), India Habitat Centre, Lodhi Road, New Delhi 110003, India

Abstract: In the present work, the effect of sodium (Na^+) and potassium (K^+) ions on the growth and fatty acid profile of marine microalgae *Dunaliella salina* was investigated. The cell growth was determined in two different phases. The growth, lipid productivity and fatty acid profile of *D.salina* were determined and compared under K^+ ion and Na^+ ion deficient and control conditions. *D.salina* under K^+ ion deficient condition exhibited higher cellular lipid content (23%) than the cells grown under control condition (20%). However, no growth was observed in Na^+ ion deficient batch. Optimum content of all fatty acid compositions (SFA = 29.39, MUFA = 20.43, PUFA = 29.15) with appropriate biodiesel properties were obtained under K^+ ion deficient conditions. The results suggests that the K^+ ion deficient condition has a significant effect on lipid content and fatty acid profile, thus *D.salina* grown in K^+ ion deficient condition holds great potential for the lipid-based biofuel production.

Keywords: Sodium, potassium, *Dunaliella salina*, growth, lipid, fatty acid.

1. INTRODUCTION

The halo tolerant species *Dunaliella salina* is a flagellate eukaryotic algae that lacks cell wall and found in saline environment exhibiting optimal growth at different salt concentrations that turn orange – red under particular culture conditions [1]. *Dunaliella salina* strains are probably the most successful microalgae in mass cultivation due to their high salinity requirements, which minimize the number of competitors and predators [2]. Presently commercial cultivation of microalgae is targeted for direct human consumption for the health food market, and for extractable compounds such as nutritional supplements or food additives (e.g. *β* carotene colouring, xanthophyll pigments like astaxanthin, and the fatty acids DHA and EPA) [3].

Smith *et al.* revealed that the uptake of K^+ into algal cells was mediated by a K^+:Na^+ symport with a 1:l stoichiometry ratio [4]. Even in higher plants (charophytes), it has been demonstrated that the uptake of K^+ in K^+ starved cells are depended on Na^+ availability.

Dunaliella cells in nature are often subjected to wide fluctuation of salt concentrations and can tolerate a broad range from low salinity to almost saturated NaCl solutions (50mM to 5M NaCl). The major mechanism of osmoregulation in this species is through production of glycerol at concentrations that are proportional to the external NaCl concentrations. In the present study, a wild strain *Dunaliella salina* was grown in sodium deficient medium and potassium deficient medium, and the growth and lipid accumulation were compared with the standardized media. The aim of this study was to check the effect of potassium and sodium ions on the lipid and fatty acid content of the species.

2. MATERIALS AND METHODS

2.1. Strain and Media

Marine microalgal strain *Dunaliella salina* was used in the present study. The strain was maintained in the growth media consisting of $MgCl_2.6H_2O$ 1.5g; $MgSO_4.7H_2O$ 0.5g; KCl 0.2g; $CaCl_2.2H_2O$ 0.26g; KNO_3 0.5g; Tris 0.6g; $FeCl_3$ 1.5mg; KH2PO4 0.1g per litre. The stock cultures were incubated at 25±2ºC with light intensity of 41µmol $m^{-2}s^{-1}$ and photoperiod of 16 hour light: 8 hour darkness in 250ml glass jars.

2.2. Experimental Setup

Batch experiments were carried out in three different media batches I) Na ion deficient II) K ion deficient III) control. Na^+ ion deficient medium was prepared by replacing the Na ions with K ions. At equimolar concentration (63.78g/l) KCl was replaced by (50g/l) NaCl. K^+ ion deficient media was prepared by replacing the K ions with Na ions, (4.2g/l) $NaNO_3$ was replaced by (5g/l) KNO3, (1.57g/l) NaCl was replaced by (2g/l) KCl and (0.22g/l) NaH2PO4 replaced by (0.2g/l) KH2PO4 and control media was prepared as mentioned in (Table **1**). All the three sets of media were added in 300ml glass jars and working volume was kept as 200ml in triplicates and autoclaved. The sterilized media was inoculated with the exponential

*Address correspondence to this author at the Biotechnology and Bioresources Division, The Energy and Resources Institute (TERI), India Habitat Centre, Lodhi Road, New Delhi 110003, India; Tel: +91118527006162; Fax: 24682144/24682145; E-mail: devivenkat.b@gmail.com

E-ISSN: 2311-8741/16 **© 2016 Savvy Science Publisher**

phase culture of *Dunaliella salina*; closed with cotton plug caps and sealed with clean wrap. Cultures were incubated under 16:8 light and dark condition at 25±2ºC and the growth was observed till 25 days (Phase I).

The cultures during the exponential phase in each jar were inoculated into 1l Erlenmeyer flask containing 800ml of medium at a proportion of 10% (v/v) and incubated under similar conditions for 25 days (Phase II). In order to avoid carry over effect the cultures were transferred from similar media (K^+ or Na^+ deficient) for at least two subcultures. To determine the growth of the cultures, samples were collected at five days interval and assayed by measuring the optical density (OD) at 680nm using Shimadzu UV-Vis spectrophotometer. Each sample was analyzed twice and the average value was calculated.

Table 1: Components and their Concentrations in Control and K^+, Na^+ Deficient Media

Components	Control	Na^+ Deficient	K^+ Deficient
KNO_3	0.2g/l	0.2g/l	-
KCl	0.5g/l	63.78g/l	-
NaCl	50g/l	-	50g/l
KH_2PO_4	0.2g/l	0.2g/l	-
$NaNO_3$	-	-	0.42g/l
NaH_2PO_4	-	-	0.22g/l

2.3. Biomass Determination

Three sets of *Dunaliella* cells were harvested by centrifugation at 7000rpm for 15 minutes [5], after completion of 25 days. Concentrated samples were washed with distilled water and dried at 45ºC for 24 hours and measured. The weighed biomass was used for lipid extraction.

2.4. Lipid Extraction and Fatty Acid Analysis

Lipid was extracted from the dried biomass according to Folch [6] method using chloroform: methanol (2:1) and water. The extract was centrifuged at 5000rpm for 10 minutes for phase separation. The upper layer (methanol – water layer) was removed and the chloroform layer containing lipid was collected. Chloroform was evaporated from extracts to yield the resultant algae lipid, which was weighed gravimetrically.

Lipid content (%) and lipid productivity (g/l/day) were calculated as follows;

Lipid content (C_{lipid}) = (wt. of lipid / wt. of sample) × 100

Lipid productivity = (C_{lipid} × DCW) / t

Where, C_{lipid} is the lipid content (%), DCW is dry cell weight (g/l) and t is the time interval (days).

Lipid extracts were converted to methyl esters with methanolic HCl and hexane. Fatty acid methyl esters (FAME) were prepared by adding 1ml of concentrated HCl with 5ml methanol. The mixture was heated at 80–90ºC in a water bath for 30min. Hexane (1ml) was added into the vial after methylation. Top hexane layer containing methyl esters were placed into GC vials for GC analysis (Agilent 6890N (USA)) equipped with a DB-5 column (0.2mm ID, 30 m, 0.25mm film by Agilent). The temperature program was started at 2ºC and was increased by 50ºC $min^{-}1$ to 250ºC. Peaks were integrated with ChemStation and identified by comparison of retention times with pure standard (Sigma). System performance was checked with blanks and standard samples prior to analysis. Concentration was expressed in mg/ml which was converted to percentage. All the tests were performed in triplicates. Degree of unsaturation is calculated to determine their potential as a biofuel on the basis of a significant biofuel property, cetane number [7].

Degree of unsaturation (DU) = MU +2 (PU)

Where, MU is the percentage of monounsaturated fatty acids and PU is the percentage of polyunsaturated fatty acids.

3. RESULTS AND DISCUSSION

3.1. Effect of Na^+/K^+ Ions on the Growth of *Dunaliella salina*

Dunaliella salina can survive in a solution containing 5mol/L NaCl and grow rapidly in the medium containing 1 to 2 mol/L NaCl [8]. The physiological mechanisms by which *D.salina* or a higher plant tolerates high Na^+ stress *via* its K^+/Na^+ selectivity [9]. In this study the complete deprivation of Na^+/K^+ ions on the growth and fatty acid profile of *Dunaliella salina* was investigated.

The growth of *Dunaliella salina* present in the media routinely used for its growth (control) and the media deficient in potassium (K ion deficient) were exhibited similar growth pattern (Figure **1**). In Na^+ deficient medium however, resulted in reduced cell division. The

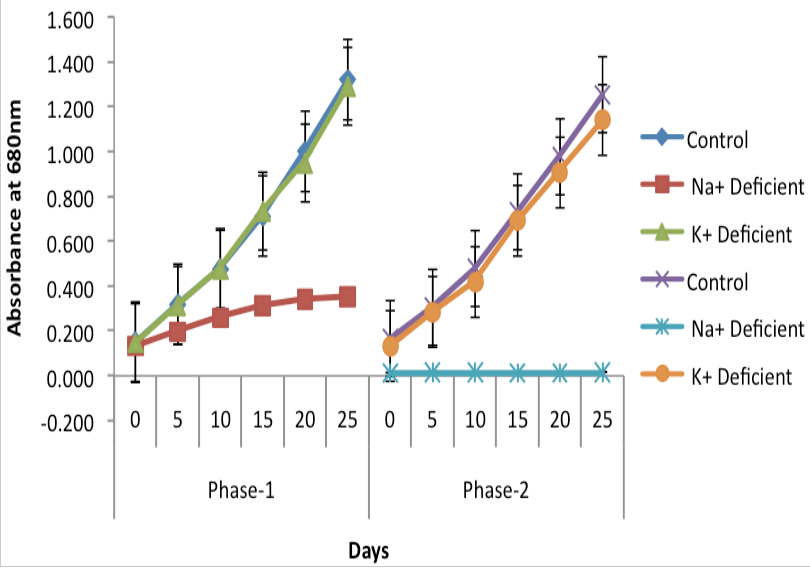

Figure 1: Growth of *D. salina* (Phase 1 and Phase 2) in three different media sets. Error bars represent standard errors (n=3).

cells showed lower cell density and entered in the decline phase much earlier (5 – 10 days). The growth of D.*salina* cells impaired due to two reasons I) absence of Na^+ ions in the media II) presence of high K^+ ions.

The sodium deprived cells lost chlorophyll and turned white. Na^+ deficiency causes impairment in photosynthesis. The defects are due to reductions in chlorophyll content, phycocyanin. Photoevolution of O_2 and ribulose bisphosphate carboxylase activity [10, 11]. The high K^+ or alkaline pH inhibits the photosynthesis of *Dunaliella salina* (Shu-Yin [9]. In our experiment, the cell growth was observed for initial 5-10 days after that the growth was declined, perhaps it is the fact that initially cells came from the routine media and it took them a while to react to the high dose of K^+. (Figure **1**)

The cultures on potassium deprived medium during the exponential phase showed biomass density and growth similar as measured that in the control. However, no growth was observed in the cells that were transferred to the fresh media of similar composition and were deprived of sodium ions.

3.2. Effect of Sodium and Potassium Ions on Lipid Content

Dunaliella salina is a marine microalgae which thrives under high NaCl. Earlier studies have demonstrated that the monovalent cations such as sodium can have a significant impact on lipid membranes. The impact of potassium ions on phospholipid membranes seems to be weaker compared to sodium ions. Similar with the results obtained in this study, exposing *D.salina* strain at K^+ ion deficient condition resulted in increasing lipid productivity as well as total lipid content than lipid obtained in control batch. The Na^+ deficient medium had practically no biomass and thus could not be evaluated for lipid (Table **2**).

Table 2: Total Biomass/Lipid Content of *Dunaliella salina* in Three Different Growth Media after 25 Days

Batches	Control	K^+ deficient	Na deficient
Biomass (g/l)	0.813 ± 0.01	0.755 ± 0.02	-
Biomass Productivity (mg/l/day)	32.52	30.2	-
Lipid content (%)	20	23	-
Lipid Productivity (g/l/day)	0.8	0.92	-

3.3 Effect of Sodium and Potassium Ions on Fatty Acid Profile

The fatty acid compositions of microalgae oil may vary to individual species/strains and their environmental conditions [12, 13]. The identification of lipid composition in selected algae strain is essential for determining the suitability of biodiesel and fuel quality. Therefore, fatty acid biosynthesis of K^+ ion deficient batch and control batch were investigated (Table **3**). The lipids of *D.salina* contained high proportion of C16 and C18 polyunsaturated fatty acids (PUFA) and noticeable amount of long chain polyunsaturated fatty acid such as (LCPUFA) C20, C22 and C24 [14].

Similarly in this study, the quantitative difference was observed in the fatty acid composition of control and K deficient grown cells. Palimitolic acid (C16:1) (8.39%), alpha linolenic acid (C18:3) (24.8%), Heptadecanoic acid (C17) (9.01%) and Arachidic acid (C20) (8.59%) were the principal fatty acids present in K deficient grown Dunaliella cells. In control medium grown cells, it was observed that there was a considerable increase in alpha linolenic acid (C18:3) (47.51%) and it has been reported in the previous studies that K^+ is necessary for the production of 18-carbon unsaturated fatty acids especially 18:3 [15]. The degree of unsaturation (DU) of the oil defined by Ramos *et al*. (2009) is an important parameter in determining the cetane number and iodine value of the final biodiesel product. Oils that have unsaturation degrees higher than 137 does not meet the European Standard for cetane number.

Table 3: Fatty Acid Profile of *Dunaliella salina* in Two Different Growth Media

Batches	K^+ Deficient	Control
C12	6.72	1.87
C14:1	1.18	1.39
C15	5.07	1.43
C15:1	4.82	1.43
C16	2.56	Nd
C16:1	8.39	10.3
C17	9.01	3.87
C17:1	0.491	1.4
C18:1	5.543	13.59
C18:2 TANS	Nd	0.723
C18:3	24.8	47.51
C18:3 (3)	1.07	2.34
C20	8.59	5.06
C22:2	2.55	7.12
Saturated	29.39	18.18
Monounsaturated	20.43	23.32
Polyunsaturated	29.15	56.97
DU	78.73	142.05
C18:1	6.72	1.87

Nd- below the limit of detection.

Higher iodine value represents more unsaturation in oil. The DU for the algal oil extracted from the K^+ deficient cells of *Dunaliella salina* was (78.73), which was lower than the control medium grown cells (142.05). Therefore K^+ deficient grown *Dunaliella salina* can be recognized as a good lipid producer for biodiesel based on the DU values.

CONCLUSIONS

Growth and fatty acid profile of *Dunaliella salina* were tested in the growth media with complete deprivation of K^+ ions and results of the study suggested that the absence of potassium ions in the growth medium did not affect the growth of *Dunaliella salina* and the lipid accumulation was comparatively higher than control batch. Owing to the special characteristic of fatty acid profile and the degree of unsaturation, K^+ ion deprivation is an ideal condition for producing biodiesel using *D.salina* strain.

ACKNOWLEDGMENTS

The authors are thankful to Department of Biotechnology (DBT) New Delhi; Govt. of India for their financial support and The Director General, TERI for infrastructure support. The authors are thankful to Nandan Singh Rawat, Laboratory Assistant, TERI for technical support.

REFERENCES

[1] Massyuk NP. Morphology, taxonomy, ecology and geographic distribution of the genus *Dunaliella Teod* and prospects for its potential utilisation. Naukova Dumka, Kiev 1973; 312.

[2] Ben-Amotz A, Shaish A and Avron M. The biotechnology of cultivating *Dunaliella* for the production of b-carotene rich algae. Bioresour Technol 1991; 38 (2-3): 233-235. http://dx.doi.org/10.1016/0960-8524(91)90160-L

[3] Wikfors GH and Ohno M. Impact of algal research in aquaculture. J Phycol 2001; 37: 968-974. http://dx.doi.org/10.1046/j.1529-8817.2001.01136.x

[4] Smith FA and Walker NA. Transport of potassium in *Charaaustralis*. I A symport with sodium. J Membr Biol 1989; 108: 125-137. http://dx.doi.org/10.1007/BF01871024

[5] Phukan MM, Chutia RS, Konwar BK and Kataki R. Microalgae Chlorella as a potential bio-energy feedstock. J Applied Energy 2011; 88: 3307-3312. http://dx.doi.org/10.1016/j.apenergy.2010.11.026

[6] Folch J, Lees M and Sloane SG. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957; 226(1): 497-509.

[7] Ramos MJ, Fernandez CM, Casas A, Rodriguez L and Perez A. Influence of fatty acid composition of raw materials on biodiesel properties. Bioresour Technol 2009; 100: 261-268. http://dx.doi.org/10.1016/j.biortech.2008.06.039

[8] Avron M and Ben-Amotz A. Dunaliella: Physiology, biochemistry, and biotechnology. Boca Raton 1992; CRC Press.

[9] Shu-Ying MA, Yang-Cheng H, Xiao-He Y and Wei-Hua WU. Effect of high K+ and alkaline pH on ultra structure of *Dunaliella salina* Chloroplasts. Acta Botanica Sinica 1999; 41: 1342-1344.

[10] Apte SK and Thomas J. Impairment of photosynthesis by sodium deficiency and its relationship to nitrogen-fixation in cyanobacterium *Anabaena torulosa*. FEMS Microbiology Letters 1983; 16: 153-157. http://dx.doi.org/10.1111/j.1574-6968.1983.tb00277.x

[11] Rai AK. Biochemical characteristics of photosynthetic response to various external salinities in halotolerant and fresh water cyanobacteria. FEMS Microbiology Letters 1990; 69: 177-180. http://dx.doi.org/10.1111/j.1574-6968.1990.tb04196.x

[12] Zittelli GC, Rodolfo L, Biondi N and Tredici MR. Productivity and photosynthetic efficiency of outdoor cultures of *Tetraselmis suecica* in annular columns. Aquaculture 2006; 261: 932-943. http://dx.doi.org/10.1016/j.aquaculture.2006.08.011

[13] Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, *et al*. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 2009; 102: 100-112. http://dx.doi.org/10.1002/bit.22033

[14] Hanaa H Abd, El-Baky K Farouk, El-Baz and Gamal S. Production of lipids rich in omega 3 fatty acids from the halotolerant alga Dunaliella salina. Biotechnology 2004; 3(1): 102-108. http://dx.doi.org/10.3923/biotech.2004.102.108

[15] Jean J Maccarthy and Gleno W Patterson. Effects of Cation Levels of the Nutrient Medium on the Biochemistry of Chlorella. Plant Physiol 1973; 54: 133-135. http://dx.doi.org/10.1104/pp.54.2.133

Received on 03-02-2016 Accepted on 10-03-2016 Published on 30-06-2016

DOI: http://dx.doi.org/10.12974/2311-8741.2016.04.01.4

© 2016 Devi and Dhawan; Licensee Savvy Science Publisher.
This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.