7.3- Effect of different nitrogen sources on Protein fractionation of *Scenedesmus obliquus*

The protein of *S. obliquus* was fractionated under optimal conditions according to the isoelectric point into the following fractions:

1. Water- soluble protein (Albumin).
2. Salt soluble protein (Globulin).
3. Alcohol soluble protein (Protamin).
4. Alkali soluble protein (Glutelin).
5. Insoluble protein.

Data presented in (Table 12 and Fig. 20) show that, different nitrogen sources affect the percentage of different protein fractions. Ammonium chloride, which recorded high protein content, elevated the content of albumin, protamin and insoluble protein fractions by (7.79%, 28.6% and 35.18%), respectively. However this is correlated by reduction in globulin and glutelin fractions by (26.3% and 46.3%), respectively. On the other hand urea as a sole nitrogen source increased albumin, globulin and insoluble protein by (6.32%, 9.4% and 111%), respectively. This is correlated by reduction of protamin and glutelin fractions by (84.9% and 70.7%), respectively.

Generally, ammonium chloride and urea induced water-soluble (Albumin) and insoluble protein fractions. Moreover NH_4Cl induced protamin (alcohol soluble fraction), while urea induced salt soluble fraction (Globulin) and Glutelin (alkali soluble protein)

The data can be summarized in the following table:

Protein fraction	**Induced by**
Water soluble protein (Albumin). Insoluble protein.	NH_4Cl- Urea
Salt soluble protein (Globulin).	Urea
Alcohol soluble protein (Protamin).	NH_4Cl
Alkali soluble protein (Glutelin)	$NaNO_3$

Table 11: Effect of different nitrogen sources on the different carbohydrate fractions (g/100g D.W) of *Scenedesmus obliquus* after 6 days of incubation.

Nitrogen source	Monosacharides	Disacharides	Polysaccharides	Total sugars
$NaNO_3$	0.31	3.6	14.19	18.1
NH_4Cl	0.42	8.6	31.58	40.6
Urea	0.86	4.20	18.1	23.16

Table 12: Effect of different nitrogen sources on the different protein fractions of *Scenedesmus obliquus* (g /100g protein) after 6 days of incubation.

Nitrogen source	Albumin	Globulin	Protamin	Glutelin	Insoluble protein
$NaNO_3$	41.1	26.6	13.3	8.2	10.8
NH_4Cl	44.3	19.6	17.1	4.4	14.6
Urea	43.7	29.1	2	2.4	22.8

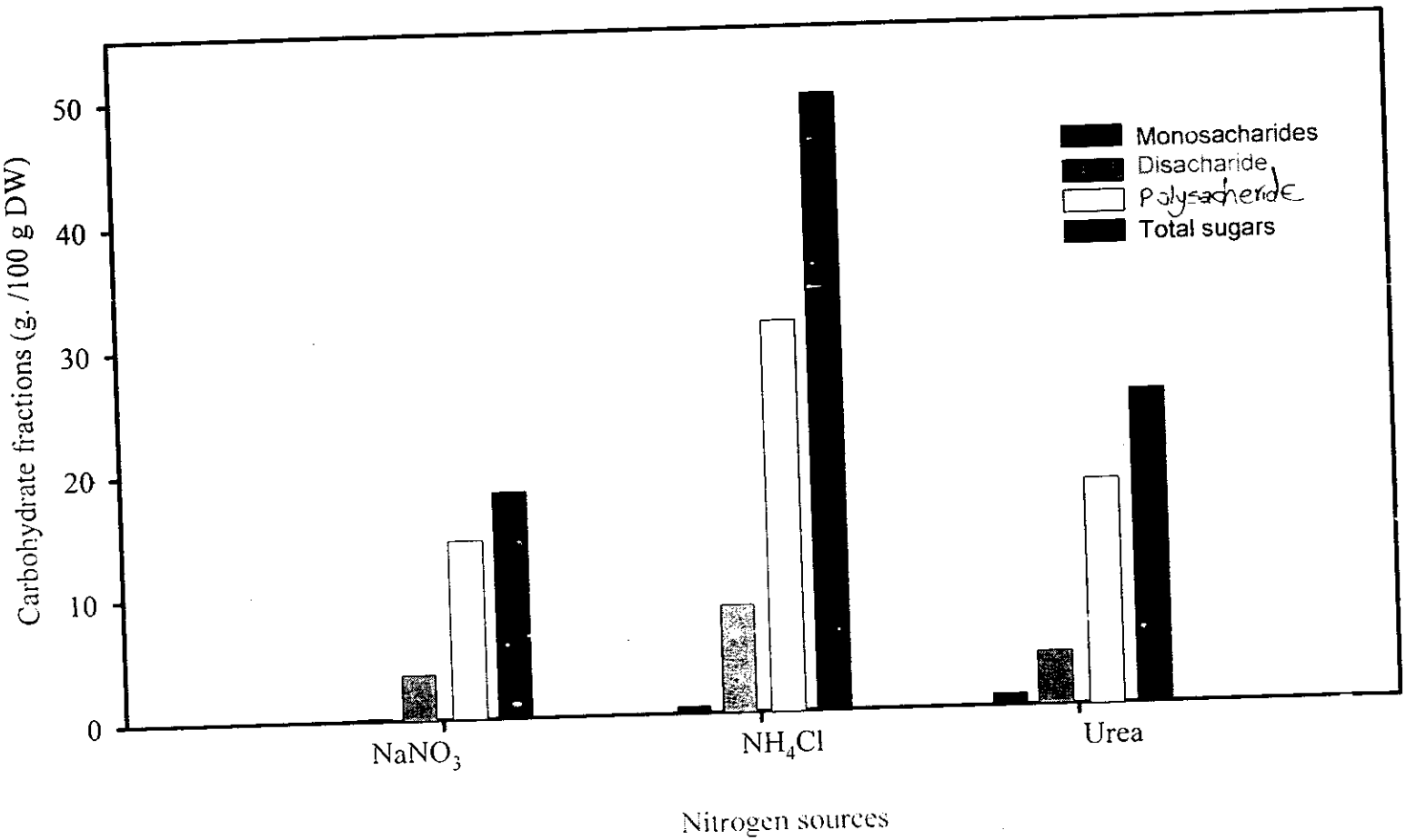

Fig. (19): Effect of different nitrogen sources on different carbohydrate fractions (monosacharides, disacharides, polysacharides and total sugars) of *Scenedesmus obliquus* after 6 days of incubation.

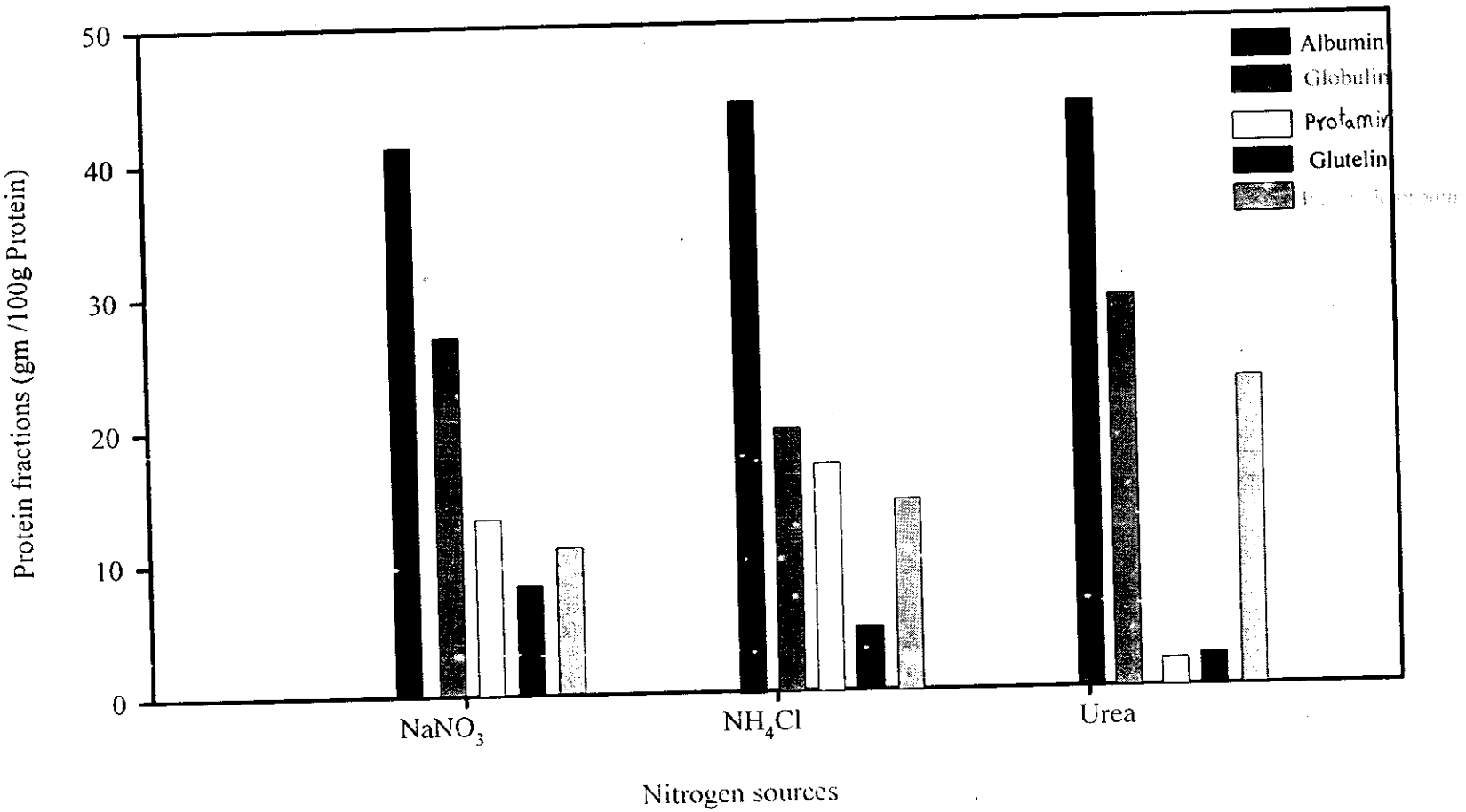

Fig. (20): Effect of different nitrogen sources on protein fractions (g/100 g Protein) of *Scenedesmus obliquus* after 6 days of incubation.

8- Effect of nitrogen starvation on growth and biochemical composition of *Scenedesmus obliquus*

Scenedesmus obliquus was incubated with nitrogen sufficient and nitrogen starved Bold media under optimum growth conditions for six days. Growth and biochemical composition parameters in terms of optical density, chlorophyll *a*, dry weight, carbohydrates, protein, lipids and nucleic acids contents were increased gradually along the experiment period (Table 13).

Optical Density: The optical density of *S. obliquus* was (1.67) at nitrogen sufficient medium, while it decreased by 45% at nitrogen starved medium (Table 13 and Fig. 21).

Chlorophyll a: Chlorophyll *a* content of *S. obliquus* was 1.17μg. ml^{-1} at nitrogen sufficient medium, while it decreased by 82% at nitrogen starved medium (Table 13 and Fig. 21).

Dry Weight: Data showed that dry weight of *S. obliquus* recorded 69 mg. l^{-1}, while it decreased by 30% at nitrogen starved medium as compared with nitrogen sufficient medium. (Table 13 and Fig. 22).

Carbohydrate content: The results in table 13 and figure 24 indicated that, carbohydrate content of *S. obliquus* recorded 234.8 mg. g^{-1} DW at nitrogen sufficient medium, while it increased by 16% at nitrogen starved medium.

Protein content: Protein content of *S. obliquus* recorded 486 mg. g^{-1} DW at nitrogen sufficient medium, while it decreased by 39.7% at nitrogen starved medium (Table 13 and Fig. 22).

Total lipids: Lipid content of *S. obliquus* recorded 8.93 mg. g^{-1} DW at nitrogen sufficient medium, while it increased by 44.6% at nitrogen starved medium (Table 13 and Fig. 22).

DNA content: *S. obliquus* recorded 0.097µg. g^{-1} DW at nitrogen sufficient medium, while it recorded lower value (0.07µg. g^{-1} DW) at nitrogen starved medium (Table 13 and Fig. 22).

RNA content: RNA content of *S. obliquus* was 0.004 µg. g^{-1} DW at nitrogen sufficient medium, while it recorded less value (0.003 µg. g^{-1} DW) at nitrogen starved medium (Table 13 and Fig. 22).

Generally, high values of growth parameters and biochemical composition determined at the end of incubation time of *S. obliquus* cultured at nitrogen sufficient medium revealed that, nitrogen is a vital nutritional element. Lipids and carbohydrate contents recorded their higher values (12.92 and 274 mg. g^{-1} DW) at starved culture of *S. obliquus*.

Table 13: Effect of nitrogen starvation on growth and biochemical composition of *Scenedesmus obliquus*. Means ± SD (n=3)

Growth parameter	*day*	$NaNO_3$	N_2-starvation
Optical density (A_{560})	*1*	0.27±0.005	0.25±0.002
	2	0.53±0.005	0.32±0.002
	3	0.80±0.010	0.58±0.007
	4	1.21±0.010	0.77±0.005
	5	1.54±0.006	0.99±0.012
	6	1.67±0.020	1.02±0.05
Chlorophyll *a* ($\mu g.\ ml^{-1}$)	*1*	0.14±0.005	0.12±0.005
	2	0.14±0.003	0.13±0.006
	3	0.33±0.002	0.18±0.003
	4	0.46±0.021	0.21±0.004
	5	0.70±0.027	0.20±0.004
	6	1.17±0.022	0.21±0.012
Dry weight ($mg.\ l^{-1}$)	*1*	11 ±2	5±1
	2	21±8	10±5
	3	39±6	29±2
	4	48±7	31±1
	5	63±32	45±8
	6	69±1	48±3
Carbohydrates ($mg.\ g^{-1}$ DW)	*1*	52±5.0	48±4.00
	2	89±4.0	72±0.64
	3	117±9.0	168±10
	4	173±19.0	226±6.0
	5	203±4.0	260±7.0
	6	234.8±3.5	274±6.3
Protein ($mg.\ g^{-1}$ DW)	*1*	189±3.0	97±4.07
	2	214±8.9	141±2.7
	3	312±5.2	182±10.7
	4	393±4.0	243±6.12
	5	451±13.0	283±18
	6	486±2.7	293±4.7
Lipids ($mg.\ g^{-1}$ DW)	*1*	0.5	0.67
	2	1.58	2.7
	3	2.60	5.33
	4	4.83	8.15
	5	7.11	9.73
	6	8.93	12.92
DNA ($\mu g.\ g^{-1}$ DW)	*1*	0.062±0.012	0.028±0.001
	2	0.067±0.0012	0.045±0.010
	3	0.077±0.012	0.057±0.001
	4	0.081±0.023	0.065±0.0050
	5	0.090 ±0.014	0.066±0.0050
	6	0.097±0.008	0.07±0.0060
RNA ($\mu g.\ g^{-1}$ DW)	*1*	0.001±0.00	0.001±0000
	2	0.002±0.0004	0.001±0000
	3	0.002±0.000	0.002±0.0004
	4	0.002±0.0003	0.002±0.0004
	5	0.002±0.00001	0.002±0000
	6	0.003±0.0002	0.004±0.001

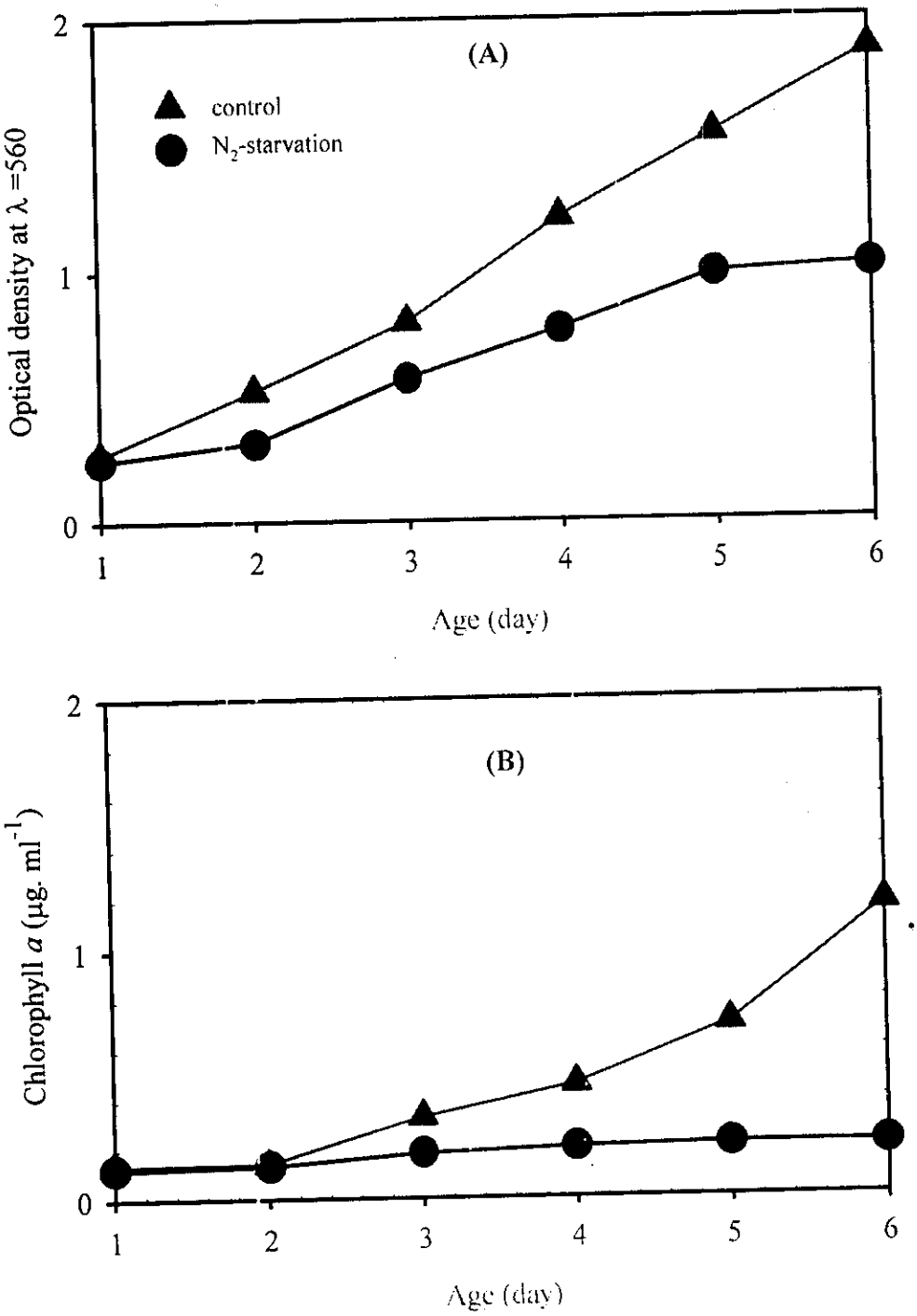

Fig. (21): Effect of nitrogen starvation on optical density (A) and chlorophyll *a* (B) of *Scenedesmus obliquus*

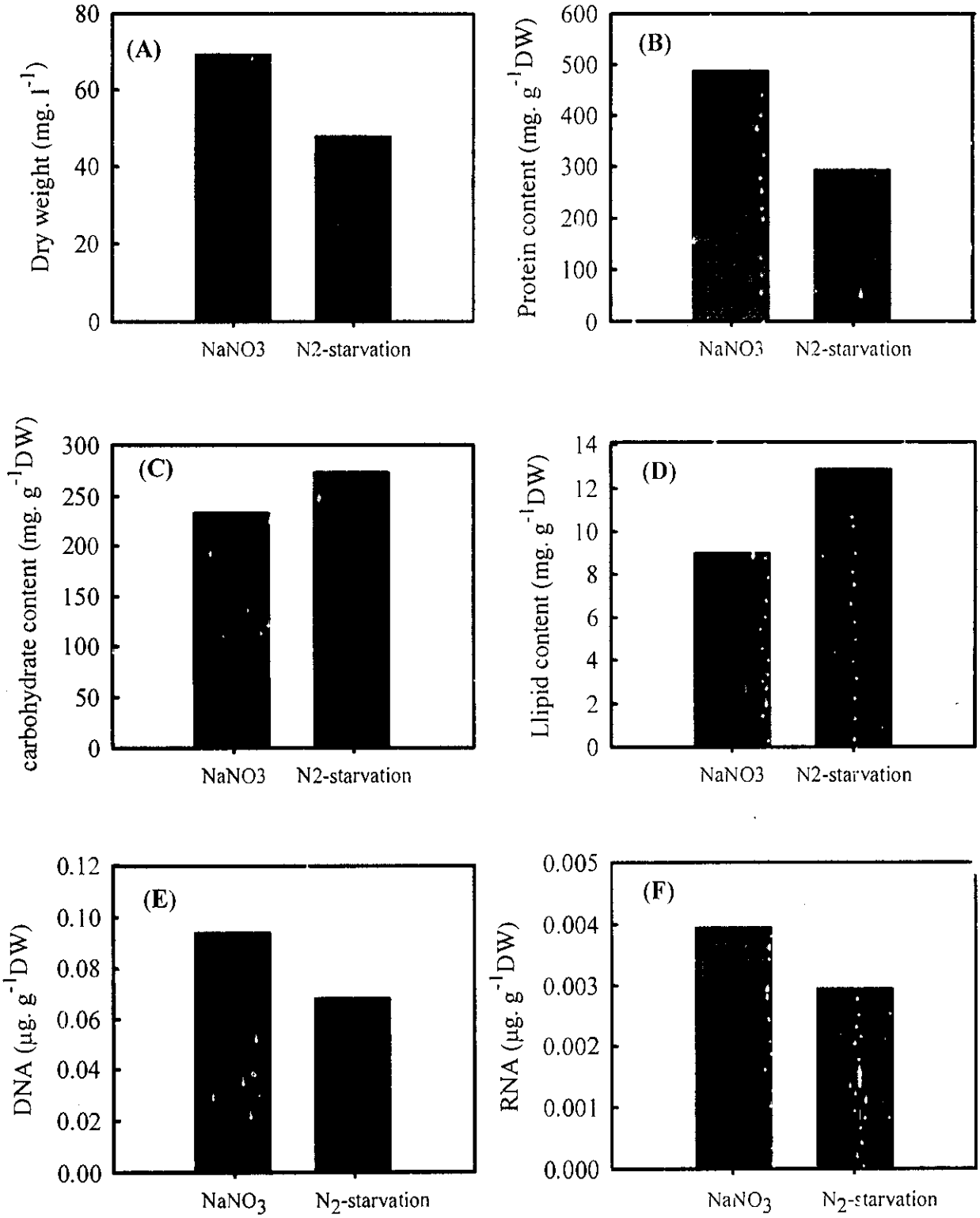

Fig. (22): Effect of nitrogen starvation on dry weight (A), protein (B), carbohydrate (C), lipid (D), DNA (E), RNA (E) contents of *Scenedesmus obliquus*.

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