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NITRITES AS A SOURCE OF NITROGEN FOR STICHOCOCCUS BACILLARIS Näg.

Contents: 1. Introduction, 2. Material and methods, 3. Results, 4. Discussion; Streszczenie; References

1. INTRODUCTION

It is generally accepted that nitrites are a source of assimilable nitrogen for algae only in concentrations not exceeding 0.001 M (14 mg N/dm^3), since greater concentrations are toxic [8, 13, 14, 15]. A number of authors have none the less attempted to cultivate algae in media containing, as the only source of nitrogen, much higher nitrite concentrations. Their results indicate clearly that some species of algae grow at least as well in such media as in the presence of nitrogen forms generally recognised as being optimal for algal growth. Thus, for example, Dunaliella tertiolecta grows well in a medium containing 35 mg N—NO_2/dm^3 [10], so does Agmenellum quadruplicatum in 138 mg N—NO_2/dm^3 [7], Scenedesmus sp. in 210 mg N—NO_2/dm^3 [2] and Anabaena cylindrica in 280 mg N—NO_2/dm^3 [3].

During studies on the application of intensive algae cultivation as a method of purifying wastes from the nitrate fertiliser industry, the filamentous Chlorophyte Stichococcus bacillaris [11] was isolated from a surge tank containing these wastes. This strain grew in media containing amonium ions, urea and nitrites, but not in media containing nitrates [12].

The object of this paper was to study the effect of various quantities of nitrite-nitrogen (N-NO_2) and the pH of the medium on the growth of S. bacillaris.

2. MATERIAL AND METHODS

Strain: Stichococcus bacillaris Näg. isolated from a surge tank containing nitrogenous wastes.

Medium: modified Prat's medium at pH 8.0 [9] containing from 5 to 2500 mg/dm³ $N\text{-}NO_2$ as the only source of nitrogen, and the same medium containing 200 mg/dm³ $N\text{-}NO_2$ at pH values from 3 to 11.

Inoculum: the medium was inoculated with S. bacillaris cells centrifuged from a 48h culture in a medium containing $(NH_4)_2SO_3$/138 mg N/dm³. The initial density of the culture was 100 ± 20 mg dry weight per litre.

Conditions of cultivation: stationary cultures of S. bacillaris were done in 500 ml glass tubes, the culture volume being 200 ml. The cultures were aerated with air enriched with 3% CO_2 and incubated for

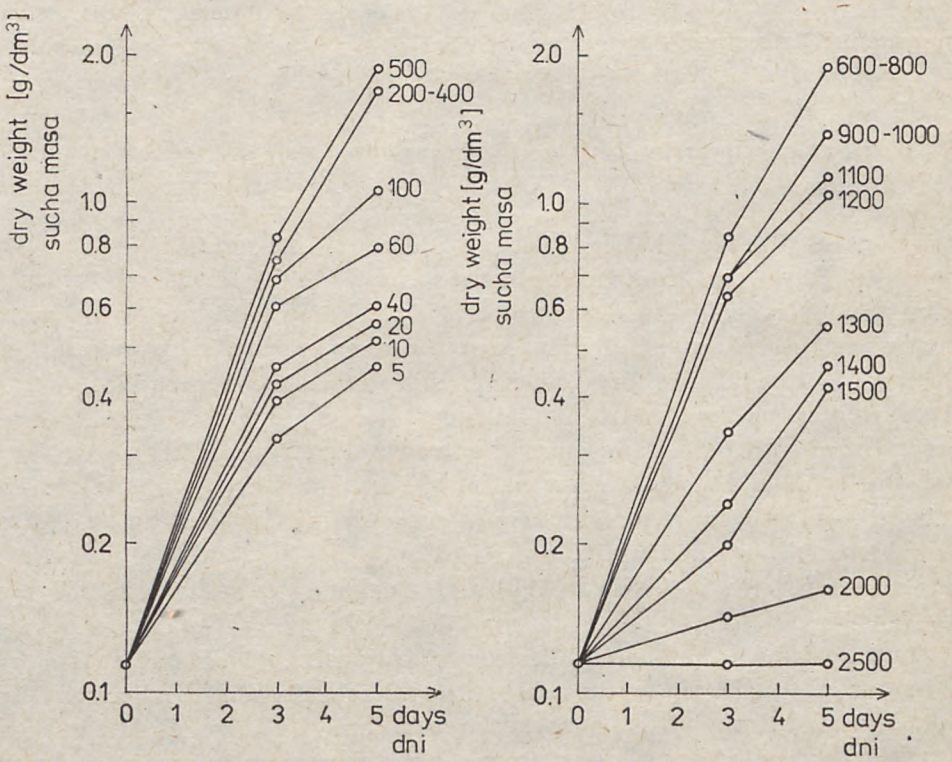

Fig. 1. Effect of nitrite nitrogen concentration in medium on dry matter production in S. bacillaris cultures
— The numbers at the end of the curves indicate concentration of $N\text{-}NO_2$ [mg/dm³]

Rys. 1. Wpływ stężenia azotu azotynowego w podłożu na produkcję suchej masy w hodowlach S. bacillaris
— Cyfry przy krzywych oznaczają stężenie $N\text{-}NO_2$ [mg/dm³]

5 days in a thermiluminostat at a light intensity of 6000 lx and a temperature of 28±2°C.

Determinations: the average cell length and the percentage of cells dividing and associated in multicellular filaments was determined by means of an immersion microscope. Dry weight was determined in a 10 ml culture sample, after centrifuging, washing and drying the algae in an aluminium foil container at 105°C to constant weight. The $N\text{-}NO_2$ content in the medium was determined using sulphanilic acid and α-naphtylamine [5], nitrogen in the algae biomass by Kjehldahl's method [5] and chlorophyll according to [4].

Every 24h, the pH was measured in all cultures and was adjusted to its initial value using 1N HCl or NaOH.

3. RESULTS

The dry weights of S. bacillaris obtained from media containing various concentrations of $N\text{-}NO_2$ are given in Fig. 1. In cultures containing from 5 to 500 mg $N\text{-}NO_2/dm^3$, the dry weight increases with the nitrogen concentration in the medium. In cultures containing from 500 to 800 mg $N\text{-}NO_2/dm^3$, the dry weight is maintained at a similar level and is around 1.8 g/dm^3 on the 5th day of incubation. In cultures containing greater nitrite concentrations, the dry weight decreases as the nitrogen concentration in the medium increases and is completely inhibited in cultures containing 2500 mg $N\text{-}NO_2/dm^3$.

Fig. 2 shows the uptake of nitrites by the growing S. bacillaris cultures. Total removal of nitrogen was found only in cultures containing 5 and 10 mg $N\text{-}NO_2/dm^3$. In those with 20 - 100 mg N/dm^3, the uptake of nitrogen with its level in the medium, although it was never removed entirely during a 5-day incubation. In the remaining cultures, between 100 and 150 mg N/dm^3 were taken up.

Table 1 gives the content of nitrogen and chlorophyll "a", and also the microscopic analysis results obtained during the growth of S. bacillaris in nitrite media. The biomass obtained from cultures in media containing from 200 to 800 mg N/dm^3 (optimum concentration for growth) contained from 5.9 to 7.4% nitrogen and from 1.2 to 1.7% chlorophyll "a". The quantities of cellular nitrogen and chlorophyll found in the biomass produced in media with a lower nitrite content were also smaller, reaching extremely low values (1.4 - 1.5% N and 0.1 - 0.2% chlorophyll) in cultures containing 5 and 10 mg $N\text{-}NO_2/dm^3$. It is also evident from the table that the nitrite content in the medium does not greatly affect the S. bacillaris cell length. The average cell length in these cultures varied from 4.2 to 6.4 μm. The nitrogen concentration in the medium did, however, markedly affect cell division.

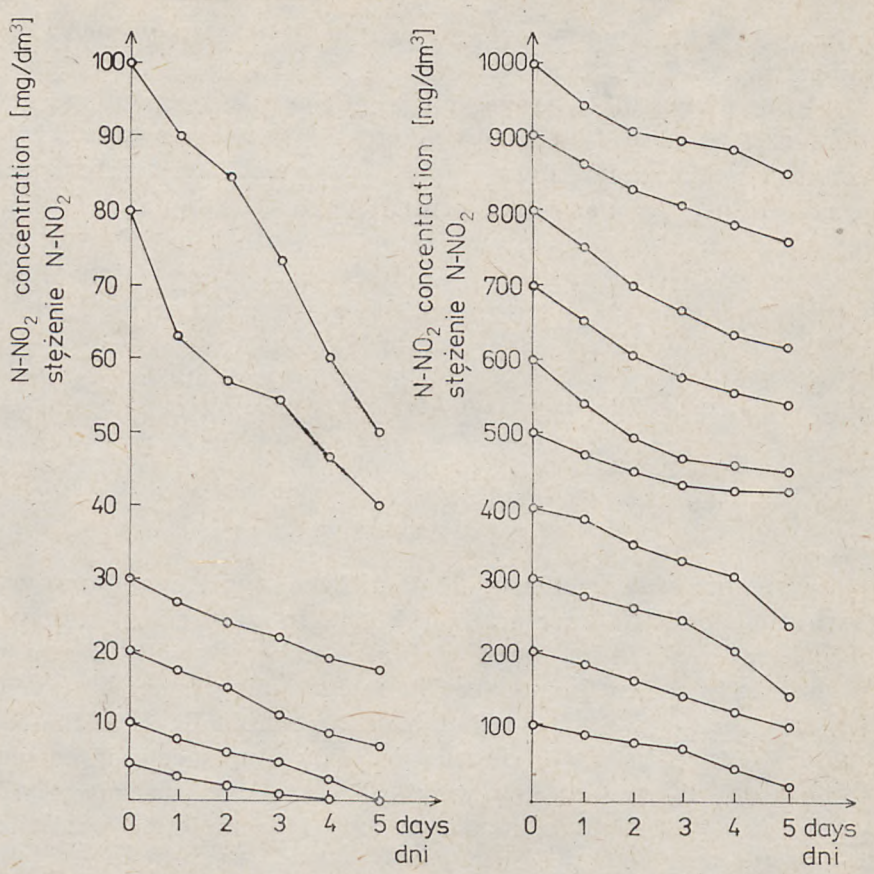

Fig. 2. Content of nitrite nitrogen in culture media found on successive days of S. bacillaris incubation

Rys. 2. Zawartość azotu azotynowego w podłożach hodowlanych stwierdzona podczas kolejnych dni inkubacji S. bacillaris

The number of cells dividing in cultures containing optimum concentrations of $N\text{-}NO_2$ is appreciably larger than in cultures containing too little or too much nitrogen. In cultures with extremely low (5 mg $N\text{-}NO_2/dm^3$) or extremely large (2000 - 2500 mg $N\text{-}NO_2/dm^3$) nitrogen concentrations, no dividing cells were found at all. In all nitrite cultures, S. bacillaris showed a distinct tendency to grow into multicellular filaments. The number of cells associated in filaments was in all cases over 50%. The highest quantity of filaments (over 97%) was found in those cultures where the quantity of nitrogen ensured maximum growth. With greater or smaller amounts of nitrogen, the number of filaments decreased with a corresponding increase in the number of single cells.

Table 2 presents the changes in pH accompanying the growth of S. bacillaris in media of various $N\text{-}NO_2$ concentrations. The greater the concentration of nitrogen in the medium, the greater its acidification

Table 1. The content of nitrogen and chlorophyll (calculated per dry weight) and cell morphology of S. bacillaris after 5-day incubation in media containing various amounts of $N\text{-}NO_2$

Tab, 1. Zawartość azotu i chlorofilu w suchej masie oraz obraz morfologiczny komórek S. bacillaris po 5-dniowej inkubacji w podłożach zawierających różne ilości $N\text{-}NO_2$

$N\text{-}NO_2$ concentration Stężenie $N\text{-}NO_2$ [mg/dm³]	Nitrogen content in dry weight Zawartość azotu w suchej masie [%]	Chlorophyll content in dry weight Zawartość chlorofilu w suchej masie [%]	Average cell length Średnia długość komórek [μm]	Percentage of cells dividing Procent komórek dzielących się	Percentage of cells associated into filaments Procent komórek połączonych w nici
5	1.5	0.1	4.2	0.0	55.9
10	1 4	0.2	5.2	2.5	70.0
20	3.4	0.2	5.5	5.0	88.9
40	3.9	0.3	6.0	8.0	87.2
80	4.1	0.2	6.2	4.0	74.6
100	5.3	0.9	6.3	5.0	91.1
200	5.9	1.3	6.0	12.4	98.0
300	7.4	1.2	6.1	16.6	97.4
400	5.9	1.3	6.1	15.6	97.4
500	6.6	1.7	5.8	18.3	99.7
600	6.7	1.5	6.4	18.6	99.6
700	7.1	1.3	6.0	20.0	97.6
800	6.0	1.2	6.1	17.6	97.4
900	6.4	1.2	6.0	13.7	91.7
1000	5.8	1.4	6.1	15.9	94.1
1100	6.4	1.5	4.9	5.5	79.5
1200	5.8	0.5	4.8	8.5	59.6
1300	6.3	0.4	4.7	3.5	69.0
1400	6.3	0.3	5.0	4.5	60.6
1500	6.0	0.3	4.9	3.0	59.0
2000	3.3	0.1	5.2	0.0	60.0
2500	4.3	0.0	5.0	0.0	56.0

during growth. The pH was adjusted daily and did not fall below 6.7 in any culture.

Fig. 3 shows the dry weight, cellular nitrogen and chlorophyll obtained in a 5-day culture of S. bacillaris in media containing 200 mg $N\text{-}NO_2/dm^3$ at various pH. It can be seen that there is a precise relationship between the pH of the nitrite medium and the growth of S. bacillaris. The increase in dry weight, cellular nitrogen and chlorophyll reached a maximum at pH 8.0. Growth was somewhat less in a neutral medium. In acidic culture conditions, no growth was found, except for cultures at pH 6.0 in which some slight increase in cellular nitrogen and dry weight was obtained, although the cells were quite colourless. With the exception of these cultures, no growth took place in acid media. Growth was much less inhibited in alkaline media (pH 9 - 11)

Table 2. The changes of pH during 5-day incubation of S. bacillaris in media containing various amounts of $N\text{-}NO_2$

Tab. 2. Zmiany odczynu podłoża w czasie 5-dniowej inkubacji S. bacillaris w podłożach z różnymi stężeniami azotu azotynowego

Concentration of $N\text{-}NO_2$ in medium Stężenie $N\text{-}NO_2$ w podłożu [mg/dm³]	pH of medium pH podłoża day of incubation dni inkubacji					
	0	1	2	3	4	5
5	8.0	6.9	7.2	7.7	7.5	7.3
10	8.0	7.0	7.2	7.6	7.4	7.4
20	8.0	7.0	7.0	7.6	7.5	7.5
40	8.0	7.0	7.1	7.9	7.6	7.9
80	8.0	7.0	7.2	7.7	7.6	7.5
100	8.0	7.0	7.0	7.8	7.6	7.6
200	8.0	7.2	7.2	7.6	7.9	7.9
300	8.0	7.3	7.4	7.8	7.9	8.0
400	8.0	7.4	7.4	7.7	7.9	7.9
500	8.0	7.3	7.4	7.8	7.9	7.9
600	8.0	7.6	7.3	7.7	7.9	7.9
700	8.0	7.4	7.4	7.7	7.9	8.0
800	8.0	7.5	7.4	7.7	7.9	7.9
900	8.0	7.4	7.3	7.8	7.9	8.0
1000	8.0	7.5	7.2	7.5	7.8	7.9
1100	8.0	7.0	7.2	7.4	7.7	7.8
1200	8.0	6.7	7.2	7.3	7.8	7.8
1300	8.0	6.7	7.9	6.9	7.5	7.5
1400	8.0	6.7	6.9	6.9	7.4	7.6
1500	8.0	6.7	6.8	6.9	7.3	7.4
2000	8.0	6.7	6.8	6.8	7.1	7.4
2500	8.0	6.7	6.7	6.7	7.1	7.3

The pH was adjusted daily to 8.0
Odczyn podłoża codziennie doprowadzano do pH 8.0

than in acid ones, although at pH 10 and 11 significantly smaller quantities of chlorophyll were found.

Table 3 shows the nitrogen and chlorophyll content in the biomass and the results of microscopic analysis during cultures of S. bacillaris in nitrite media (200 mg $N\text{-}NO_2/dm^3$) at various pH. In cultures at pH 8, the biomass contained 6.5% nitrogen and 1.9% chlorophyll. In the remaining cultures, the nitrogen and chlorophyll content in the biomass was lower, whereby the chlorophyll content decreased more rapidly than the nitrogen content. The pH of the medium did not greatly affect the average length, although cells were distinctly smaller in very acid media (pH 3.0) and distinctly larger in very alkaline media (pH 11.0) than in the other cultres. The pH of the nitrite-containing media had a much greater influence on cell division and the quantity of multi-

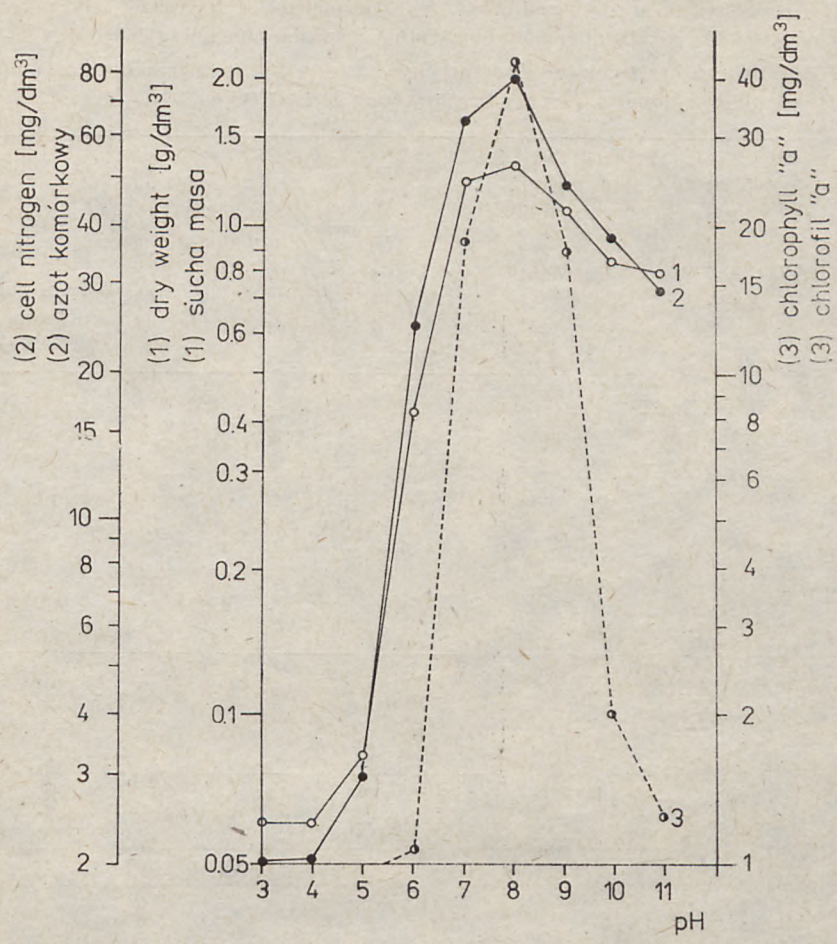

Fig. 3. Dry weight, cellular nitrogen and chlorophyll content obtained in cultures of S. bacillaris on the 5th day of incubation in nitrite medium (200 mg N/dm³) at various pH (3.0 - 11.0).

Rys. 3. Ilość suchej masy, azotu komórkowego i chlorofilu uzyskana w hodowlach S. bacillaris w 5 dniu inkubacji na podłożu azotynowym (200 mg/dm³) przy różnym pH (3.0 - 11.0)

cellular filaments. There was no cell division in cultures at pH 3.0 - 5.0; the largest number of dividing cells was found at pH 8.0 (15%); in highly alkaline media (pH 11.0) 8% of cells were found to be dividing. Over 95% of cells were associated filaments in cultures at pH 7.0 - 10.0 — whereas in other cultures, there were more single cells; however, in no culture did the quantity of filaments fall below 65%.

Growth of S. bacillaris in nitrite cultures (200 mg $N\text{-}NO_2/dm^3$) was accompained by changes in pH in the medium (Fig. 4). There was a definite tendency for acid media to become alkaline and vice versa. pH changes in neutral or weakly alkaline cultures were slight.

Table 3. The content of nitrogen and chlorophyll (calculated per dry weight) and microscopic analysis of S. bacillaris cells after 5-day incubation in media containing 200 mg $N\text{-}NO_2/dm^3$

Tab. 3. Zawartość azotu i chlorofilu w suchej masie oraz obraz mikroskopowy komórek S. bacillaris po 5-dniowej inkubacji na podłożach zawierających 200 mg $N\text{-}NO_2/dm^3$

pH	Nitrogen content in dry weight Zawartość azotu w suchej masie [%]	Chlorophyll content in dry weight Zawartość chlorofilu w suchej masie [%]	Average cell size Średnia wielkość komórek [μm]	Percentage of cells dividing Procent komórek dzielących się	Percentage of cells associated into filaments Procent komórek połączonych w nici
3.0	2.0	0.0	3.8	0.0	73.0
4.0	3.3	0.0	4.0	0.0	74.5
5.0	3.8	0.0	4.2	0.0	78.2
6.0	6.0	0.3	4.4	3.0	79.4
7.0	5.5	1.5	4.8	10.0	98.5
8.0	6.3	1.9	4.7	15.0	99.1
9.0	4.6	1.3	4.7	10.0	96.8
10.0	4.5	0.2	4.9	12.0	95.7
11.0	3.5	0.1	5.5	8.0	65.7

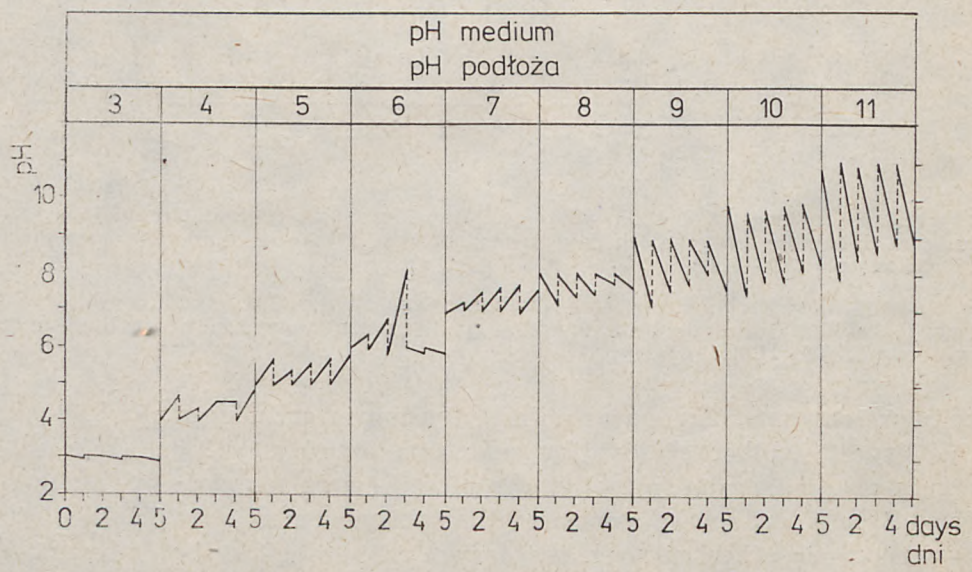

Fig. 4. Changes of the pH value accompanying growth of S. bacillaris in nitrite media (200 mg N/dm^3) at various pH (3.0 - 11.0). The broken line indicates the adjustment of pH to initial value.

Rys. 4. Zmiany pH towarzyszące wzrostowi S. bacillaris w podłożach z azotynami (200 mg/dm^3) o różnym odczynie (pH 3.0 - 11.0). Linią przerywaną oznaczono regulację pH podłoża do wartości początkowej.

4. DISCUSSION

The results presented in this paper contradict the widely held opinion that only very small concentrations of nitrites can be a useful source of nitrogen for algae. They also contradict the results of Tomova et al. [15] who found that Chlorella vulgaris takes up nitrities if their concentration is 14 mg N/dm^3, but not at concentrations of 140 mg N/dm^3.

As has been shown, S. bacillaris does not grow well in media with a low concentration of nitrites. The biomass from such cultures contains little nitrogen and chlorophyll, the cells are much smaller and the number of dividing cells decreases. This phenomenon seems to suggest nitrogen deficiency, despite the fact that in cultures where the nitrite content is greater than 10 mg N/dm^3, some of the nitrogen remains in the medium to the end of incubation. Optimum growth of S. bacillaris is obtained in media containing from 200 to 800 mg N-NO_2/dm^3. This concentration is about the same as the optimum concentration of urea and greater than that of ammonium ions [12].

It appears that the reason for the discrepancy between the general opinion that high concentrations of nitrite nitrogen are toxic to algae and the results obtained in this study lies in the fact that S. bacillaris grows in media at pH 8.0. This pH was used because it was the optimum for S. bacillaris growth in cultures containing other sources of nitrogen [12].

The effect of pH on the growth of algae in nitrite cultures has already been studied by Bongers [2]. This author found that in media containing 210 mg N-NO_2/dm^3 the growth intensity was similar to that in media containing identical concentrations of ammonium and nitrate ions, providing that the pH was neutral or alkaline. No growth was found in acid media (pH 2.0 - 6.0), and the gradual disappearance of chlorophyll in the cells was observed.

The results obtained with S. bacillaris cultures in media containing 200 mg N-NO_2/dm^3 at various pH (3.0 - 11.0) entirely confirm the results obtained for Scenedesmus sp. [2].

The lack of growth of S. bacillaris in nitrite media at pH 3.0 - 6.0 was probably the result of HNO_2 formation in these cultures. In an acid environment, there is a shift in the equilibrium between NO_2^- and HNO_2 favouring the production of undissociated nitrous acid. The nomograms given by Anthonisen et al. [1] show that 16 mg HNO_2 are formed in a medium containing 200 mg N-NO_2 at pH 5.0. Such a quantity of nitrous acid must affect S. bacillaris since, as Hiller and Bassham [6] state, concentrations as low as 2.3 mg/dm^3 will irreversibly inhibit photosynthesis in Chlorella pyrenoidosa.

The results of this study indicate that nitrites could be as good

a source of nitrogen for algae as urea, nitrates or ammonium ions, provided that cultures are done in neutral or slightly alkaline media, and that over-rapid acidification of the medium is prevented.

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AZOTYNY JAKO ŹRÓDŁO AZOTU DLA STICHOCOCCUS BACILLARIS NÄG.

Streszczenie

Badano rozwój Stichococcus bacillaris Näg. w podłożach zawierających od 5 do 2500 mg N—NO_2/dm³ przy pH 8.0. Wykazano, że S. bacillaris rośnie słabo w podłożach z niskim stężeniem azotynów, biomasa tych hodowli zawierała niewiele azotu i chlorofilu, komórki były znacznie mniejsze, a także spadała liczba komórek dzielących się.

Stwierdzono, że optymalne dla wzrostu szczepu stężenie azotu wynosi od 200 do 800 mg/dm³. Zmieniając wartości pH od 3.0 do 11.0 w hodowlach na podłożu zawierającym 200 mg N—NO_2/dm³ ustalono, że wzrost badanego szczepu jest możliwy w tych warunkach jedynie przy obojętnym lub alkalicznym odczynie podłoża.

Brak wzrostu S. bacillaris na podłożu zawierającym azotyny, przy wartości 3.0 - 6.0 pH, był najprawdopodobniej spowodowany tworzeniem się w tych warunkach HNO_2.

Generalnie, na podstawie wyników uzyskanych w pracy niniejszej, można wnioskować, że azotyny, przy zachowaniu odpowiedniego pH podłoża, mogą stanowić dla glonów równie dobre źródło azotu, jak mocznik, azotany czy amon.

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