Andrzej SUROWIEC, Wacław ZAWADZKI,
Tadeusz WILCZOK

Silesian Medical Academy
Institute of Chemistry and Physics — Sosnowiec

Oceanologia, 17 (1983)
PL ISSN 0078-3234

STUDY OF TRITIUM RETENTION IN CHLORELLA PYRENOIDOSA AND SCENEDESMUS ACUTUS CELLS

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

1. INTRODUCTION

Tritium present in the environment originates from three main sources i.e. the atmosphere, nuclear weapon tests and nuclear power reactors. The main physical parameters of this nuclide are: 100% β-radiation, relatively long half-life (12.3 years), maximum energy of 18.6 keV and average energy of 5.7 keV [8]. The development of the nuclear power, following present trends will create large potential sources of tritium released into the environment in increasing quantities. Apart from tritium, iodide isotopes, krypton, xenon and carbon are sometimes the main nuclear power waste products called "mobility environmental" nuclides [3, 5].

The average annual value of tritium released to the biosphere was $4.05 \cdot 10^2$ TBq (10.95 kCi), with 35 TBq (0.95 kCi) liquid waste (Douglas Point, Canada 1970) [5] and for the same type of reactor in fuel reprocessing plants (annual capacity of uranium 450 matric tons), the tritium release amounts to $7.03 \cdot 10^4$ TBq (1.9 MCi) [12]. Assuming that the nuclear power engineering based on fission reaction will progress according to the accepted programmes, the amount of tritium will reach $2.6 \cdot 10^7$ TBq (700 MCi) by the year 2000 [5].

The detailed evaluation carried out by the International Atomic Energy Agency [5], indicates that the assumed amounts of tritium released will not constitute a hazard to human populations. We would like, however, to give some facts concerning the presence of tritium in the environment, which may change the approach to this problem.

First, the development and use of thermonuclear reactors i.e. reaction generation, necessitates the employment of large amounts of tritium for fusion reaction. Steiner and Fraas [13] estimated the tritium balance at about $2.2 \cdot 10^6$ TBq (60 MCi) per 1000 MW capacity and according to Crowson quoted by Szot and Rochalska [15], a 3000 MW thermonuclear reactor will produce $3.7 \cdot 10^5$ TBq (10 MCi) of tritium daily. In consequence, the tritium production in fusion reaction will be $10^3 - 10^5$ times higher than in existing fission reactors of the same capacity.

Secondly, ecological data which will enable the estimation of population exposure to tritium through food chains and that firmly bound to some basic chemical compounds, are needed.

Thirdly, pulse release of tritium into the environment may occur in the case of accidents. Recently, for example, such a case took place at Three Mile Island [16], when some tritium and other isotopes were released to the biosphere.

Most tritium is released in the form of tritiated water (HTO) and to a lesser degree as elemental HTO to the sea or rivers as low-level waste. Because of the chemical resemblance of HTO to water, the removal of this nuclide from liquid waste may be impossible and to date HTO passes through the most sophisticated demineralisers [16]. Since algae and some plants constitute the beginning of the food chain, the examination of tritium incorporation and retention by these organisms is essential. Determination of tritium retention in basic chemical compounds of algae resulting from contamination of the hydrosphere seems to be particularly important. Experiments with Spirulina platensis algae cultivated in a medium containing HTO and then transferred to a non-active medium, showed that algae compounds contain firmly-bound tritium [14].

The determination of the extent and degree of tritium retention, due to its close connection with the problem of radiation protection is of great importance because of the exposure of the population throughout the food chain [1, 7].

The aim of the studies was comparison of tritium incorporation and retention in Chlorella pyrenoidosa and Scenedesmus acutus cultures under the same physical conditions and initial HTO concentration.

2. MATERIAL AND METHODS

Two algae species, Chlorella pyrenoidosa and Scenedesmus acutus, were cultivated separately in a liquid medium after Lefévre [9]. 500 ml flasks, containing 165 ml of medium were continuously iluminated by means of glow-tube lamps at an intensity of 8000 lx. A constant temperature of 28°C was mantained during the whole period of cultivation (14 days).

At the very beginning of the cultivation, before algal inoculation, a few millilitres of tritiated water of given activity were added to the medium in order to obtain an initial radioactivity of medium equal to 2.2 Bq/ml (6 mCi/100 ml). Control cultures without HTO were cultivated simultaneously. Each culture was occasionally agitated using magnetic stirrers. The experiment ran as follows:

One-third of the algal suspension was centrifuged (1300 xg; 10 min), washed four times with non-active (non-radioactive) medium, and centrifuged after each washing. The residual volume of the culture was centrifuged, washed four times with non-active medium, also centrifuged after each washing, and the sediment of washed algae suspended in non-active medium of a volume equal to that of the culture from which the given amount of algae originated, then cultivated for 60 hours. Afterwards, one-third the volume of this culture was centrifuged and washed with non-active medium, the remaining two-thirds, after centrifugation and washing, being suspended in a proportional volume of non-active medium and cultivated for a further 60 hours. This procedure was repeated twice, then the cultivation was terminated.

The following samples of algae were obtained from the successive stages of experiment:

— after cultivation in radioactive tritium medium
— after 1st retention (60 hrs after removal of tritium from medium)
— after 2nd retention (120 hrs after removal of tritium from medium)
— after 3rd retention (180 hrs after removal of tritium from medium).

Radioactivity of the algae was determined as the sum of radioactivities of each fraction obtained during the isolation of the main chemical cell compounds, according to Kern's method with Wanka's modifications [17]. Details of the method are given elsewhere [14]. This method of determination of the total algal radioactivity ensures higher credibility of results as compared with the method of direct counting of activity of all the cells placed in the scintillator. In the latter method part of the tritium radiation energy is absorbed by the relatively thick cell wall of the algae (the cell wall is about 0.3 μm thick; average tritium β-radiation range in water is about 2 μm).

The applied methods of desintegration of the cells were ineffective because of their low lytic action (e.g. Protosol, New England Nuclear, USA) or because of the possibility of the chemiluminescence effect which gives additional counts, irrelevant to the ionizing radiation (e.g. H_2SO_4). Furthermore, using Kern's method with Wanka's modification we could determine the radioactivity of cell water and that of dry matter (organic fraction) separately. The radioactivity of the cell water was assessed as being equal to the difference between the 0.2 N PCA fraction radioactivity in Kern's method and the RNA radioactivity as given in paper [14].

The protein contents, determined by Lowry's method [10], was help-

ful when assessing the amounts of wet and dry mater of the algae. The absorption dose rate was estimated according to its definition using the basic equation:

$$\text{Dose rate [rad/day]} = \frac{A \cdot \bar{E} \cdot k \cdot 1.44 \cdot 10^3}{100}$$

where: A is the specific radioactivity in dpm per 1 g of mass
$\bar{E}=5.69$ keV is the mean value of energy for beta radiation of tritium
$k=1.602\ 10^{-9}$ ergs/keV
100 is the ergs/g to rad conversion coefficient.

Radioactive samples were counted by means of a Packard Tri-Carb 3385 Liquid Scintillation Spectrometer using a scintillator based on dioxane, as given in paper [14]. Counting time was so chosen that the standard error in a given sample count would never exceed 5%.

3. RESULTS AND DISCUSSION

The experiment aimed to simulate HTO pulse discharge to the aquatic environment resulting in tritium incorporation into algal cells. The labelled biomass transfer to non-active medium models natural decontamination of this environment, to a certain extent.

The results of the experiments show that the tritium content in the cells of the algal species analysed falls after radioactive biomass transfer

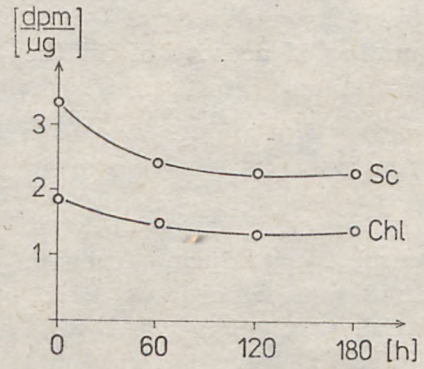

Fig. 1. Specific radioactivity of the whole algae cells in function of retention time
Rys. 1. Radioaktywność właściwa całych komórek glonów w funkcji czasu retencji

to non-radioactive medium. Specific radioactivities proper to whole cells in the retention process over 180 h in a medium without HTO are equal to 70% and 75% of the initial radioactivity for S. acutus and Ch. pyrenoidosa respectively, see Fig. 1. It was observed that after keeping

Spirulina platensis algae cells in non-radioactive medium for 60 h they showed a greater decrease in radioactivity, equal to 25.5% of their initial radioactivity [14].

This expected effect is connected with the presence of chemical components with tritium incorporation properties during biosynthesis [6] in positions in which $^3H \rightarrow H$ exchange may occur.

It is also known that tritium uptake from the medium is discriminated in favour of protium [14, 11]. One can expect that the amount of tritium built in to algae cells, after termination of the cultivation in radioactive medium was greater than that presented in Fig. 1 (time "0"). Tritium

Table 1. Radioactivity ratios of both algal species analysed

Tab. 1. Stosunki radioaktywności analizowanych gatunków glonów

Sample obtained after Próbka otrzymana po	Chlorella pyrenoidosa Radioactivity ratio of			Scenedesmus acutus Radioactivity ratio of		
	„A" dry matter whole cells sucha masa cała komórka	„B" cell water whole cells woda komórkowa cała komórka	„A" „B"	„A" dry matter whole cells sucha masa cała komórka	„B" cell water whole cells woda komórkowa cała komórka	„A" „B"
cultivating in tritium medium hodowli w radioaktywnym medium	3.47	0.38	9.13	4.04	0.24	16.8
1st change of medium pierwszej zmianie pożywki	2.67	0.33	8.09	3.07	0.16	19.2
2nd change of medium drugiej zmianie pożywki	2.56	0.27	9.48	2.94	0.13	22.6
3rd change of medium trzeciej zmianie pożywki	2.62	0.29	9.03	3.04	0.12	25.3

must have passed to the rinsing medium during the processes of cell separation from the base and washing (see Material and methods). Several washings were necessary to remove HTO adsorbed on cells and so that the tritium activity in cell water fraction would originate solely from this source. The effect to the rapid tritium excretion was not the subject of this experiment. The drop in radioactivity observed in 0 - 60 h interval, see Fig. 1, results mainly from $^3H \rightarrow H$ exchange in structures in which the exchange process has longer time constants.

An important observation from the point of view of radioecology is

that some tritium is relatively permanently bound and it does not undergo any exchange despite further changes in the cultivation medium.

The ratio values of the radioactivity of dry matter and cell water to the whole cells, as well as organic fraction/cell water ratios are presented in Table 1. Comparision of the results shows that the tritium content in the organic fraction of dry mass is on the average 9 times higher than in cell water for Chlorella algae, irrespective of time. The analogical values for S. acutus algae are higher and amount to 16.8 - - 25.3 with the additional tendency to increase whilst kept in non-active medium. Bogen and Welford [2] proved that the ratio of specific radioactivity of organic fraction to water fraction depends on the trophic level and for the soil, e.g. amounts to 6 - 8, vegetation 3 - 4 and fish 2.4.

The ratio values obtained in our experiments for Ch. pyrenoidosa algae are within the range of those for soil, see Table 1. This means that steady radioactivity level starting from 60 h is primarily connected with this nuclide in all chemical components of algae apart from water. Earlier studies [14] with S. platensis showed that lipids and assimilative pigment fraction contains the largest amount of tritium. It should also be stressed that the percentage contribution of this nuclide in these compounds during cultivation in non-radioactive medium increased.

Similarly, it seems that the main cause of tritium retention in Ch. pyrenoidosa and S. acutus algae cells is permanent binding of this nuclide with pigments and lipids. Nucleic acids and proteins contribute little to the retention effect [14].

Comparision of the data in Table 1 for the two species of algae shows that tritium retention is higher in Scenedesmus algae. Similarly, a higher percentage of this nuclide is observed in the dry matter of

Table 2. Absorption dose rate of algal dry matter, cell water and whole cells in the function of retention time

Tab. 2. Moc dawki absorpcyjnej dla suchej masy, wody komórkowej i całych komórek glonów w funkcji czasu retencji

Time of retention [hrs] Czas retencji [godziny]	Dose rate in initial medium [rad/day] Moc dawki wyjściowej [rad/dzień]	Chlorella pyrenoidosa dose rate [rad/day] moc dawki [rad/dzień]			Scenedesmus acutus dose rate [rad/day] moc dawki [rad/dzień]		
		whole cells cała komórka	dry matter sucha masa	cell water woda komórkowa	whole cells cała komórka	dry matter sucha masa	cell water woda komórkowa
0	17.1	0.24	0.85	0.093	0.43	1.74	0.105
60		0.19	0.65	0.081	0.32	1.32	0.072
120		0.17	0.62	0.066	0.30	1.27	0.059
180		0.18	0.64	0.072	0.30	1.31	0.051

Scenedesmus cells than in the Chlorella algae of the species investigated. More efficient tritium labelling of Scenedesmus algae biomass than in Chlorella algae, as well as a higher level of this nuclide after its removal from the culture involves some radioecological implications. In other words, the presence of Scenedesmus cells in the hydrosphere considered and their contamination by the HTO present in this environment, may cause a potentially greater threat of the transfer of larger amounts of tritium to higher organisms than would take place in the case of Chlorella algae.

The results concerning the dose rate estimation of tritium β-rays according to the formula given in "Material and methods" are shown in Table 2.

Although the radiation doses for algal dry matter are significantly higher than those for cell water, these values should generally be considered as being low. Furcinitti and Todd [4] recently proved that even low-level radiation will produce some genetic damage in cultures of human kidney cells. They found, for example, that 21 rad doses killed some cells. It results from this, that even relatively low tritium incorporation into algae and vegetation, which constitute the beginning of the human food chain, can not be ignored if linear relation between dose and morbidity rate is valid. In accordance with the above-mentioned "linear hypothesis", all doses have some cancer-inducing effect.

Andrzej SUROWIEC, Wacław ZAWADZKI, Tadeusz WILCZOK

Śląska Akademia Medyczna
Instytut Chemii i Fizyki — Sosnowiec

BADANIA RETENCJI TRYTU W KOMÓRKACH GLONÓW CHLORELLA PYRENOIDOSA I SCENEDESMUS ACUTUS

Streszczenie

Przeprowadzono porównawcze badania inkorporacji trytu do komórek hodowanych w obecności HTO oraz retencji tego nuklidu po usunięciu HTO z pożywki dla dwóch gatunków glonów: Chlorella pyrenoidosa oraz Scenedesmus acutus. Radioaktywność właściwa komórek zmniejsza się pod koniec obserwowanego procesu retencji (180 h) do 70 - 75% wartości początkowej. Stwierdzono dużo większą zawartość trytu w suchej masie w stosunku do zawartości w wodzie komórkowej oraz wykazano znaczne różnice ilościowe dotyczące tych gatunków glonów.

Stosunek radioaktywności frakcji organicznej do wody komórkowej jest dla Ch. pyrenoidosa niezależny od czasu i wynosi w przybliżeniu 9, natomiast dla S. acutus w trakcie 180-godzinnej retencji wzrasta od 16,8 do 25,3.

REFERENCES

1. Bittle R., R. Kirchmann, G. Van Gelder-Bonnijs, G. Koch, *Etude d'un ecosysteme aquatique naturel contaminé in situ par des effluents liquides trities, en vue de l'évaluation de la sensibilité des parameters des niveaux d'exposition du public*, in: *Population dose evaluation and standards for man and his environment*, IAEA, 1974.
2. Bogen D. C., G. A. Welford, *"Fallout tritium" distribution in the environment*, Health Physics, 30, 1976.
3. Bond V. P., *Long-lived isotopes arising from nuclear power production: ^{3}H and ^{85}Kr*, in: *Human and ecologic effects of Nuclear Power Plants*, L. A. Sagan (Ed.), 1974.
4. Furcinitti P. S., P. Todd, *Gamma rays: Further evidence for lack of a threshold dose for lethality to human cells*, Science, 206, 1979.
5. IAEA., *Nuclear power and the environment*. Polish transl. *Energetyka jądrowa a środowisko*, Warszawa 1975.
6. Kanazawa T., K. Kanazawa, J. A. Bassham, *Tritium incorporation in the metabolism of Chlorella pyrenoidosa*, Environ. Sci. Technol., 6, 1972, 7.
7. Kirchmann R., J. Van den Hock, G. Koch, V. Adam, *Studies on the food chain contamination by tritium*, in: *Tritium* (Moghissi A. A., Carter M. W., Eds.), Messenger Graphics, Phoenix 1973.
8. Lederer C. M., J. M. Hollander, I. Perlman, *Table of isotopes*, 6th ed., John Wiley and Sons Inc., New York 1967.
9. Lefévre M., H. Jacob, M. Nisbet, *Auto- et hétéroantagonisme chez les algues d'eau douce in vitro et dans les collections d'eau naturelles*, Ann. Stat. Centr. d'Hydrobiol. Appl. 4, 1952.
10. Lowry J. O. H., N. J. Rosenbrough, A. L. Farr, R. J. Randall, *Protein measurement with the Folin-phenol reagent*, J. Biol. Chem., 193, 1951.
11. Rambeck W. A., J. A. Bassham, *Tritium incorporation and retention in photosynthesizing algae*, Biochimica et Biophysica Acta, 304, 1973.
12. Rohwer P. S., W. H. Wilcox, *Radiological aspects of environmental tritium*, Nuclear Safety, 17, 1976, 2.
13. Steiner D., A. P. Fraas, *Preliminary observations on the radiological implications of fusion power*, Nuclear Safety, 13, 1972, 5.
14. Surowiec A., W. Zawadzki, M. Sosnowska, *Radioactivity of nucleic acids and protein of Spirulina platensis algae cultivated in medium containing HTO*, Nukleonika, 24, 1979, 10 - 11.
15. Szot Z., M. Rochalska, *Tryt. Metabolizm i skutki biologiczne*, Warszawa 1979.
16. Torrey L., *Three Mile Island — the lessons*, New Scientist, 84, 1979, 11803.
17. Wanka F., *Die Bestimmung der Nukleinsäuren in Chlorella-Kulturen*, Planta (Berl.) 58, 1962.