Bull. Inst. Oceanogr. & Fish., ARE, 13 (2), 1987: 43 - 51.

DIFFUSION UPTAKE OF AMINO ACIDS IN SHALLOW WATERS

WAGDY M. EL-SARRAF*, M.A. ABDELMONEIM* AND J. OLAH**.

*National Institute of Oceanography and Fisheries, Anfoushy, Alexandria, Egypt.
**Fish Culture Research Institute, H - 5541 Szarvas, Hungary.

ABSTRACT

During the determination of heterotrophic potential, it was observed that the results did not agree with Michaelis - Menten kinetics but rether followed simple diffusion in water bodies. Simple diffusion occurred at different temperatures at both high and low substrate concentrations. The results differ than previous ones by the fact that the simple diffusion occurred at concentrations higher than 500 µg dm^{-3}. According to this observation, it can be assumed that dissolved amino acids may serve as an additional carbon or nitrogen source and , only under a very limited conditions, also as an energy source.

INTRODUCTION

Dissolved organic matter is an important energy source for bacteria and other microheterotrophs in water. Fogg (1966), focused consideration on the phytoplankton as a primary source of dissolved organic matter in natural waters. The growth of phytoplankton depends on the supply of nitrogen which is a limiting nutrient. Under unfavourable conditions, the growth of phytoplankton is affected by dissolved organic matter in the environment. It should be noted that many species of phytoplankton are occasionally heterotrophic. The ability to grow heterotrophically has been reported for species from all main divisions of algae (Parsons and Strickland, 1962). Also, microflagellata are capable of prolonged growth in complete darkness (Wood, 1959). Wright and Hobbie (1966) suggested that two mechanisms of uptake are present, the one measured at the lower substrate concentration was due to bacteria (first-order kinetics), and the other, at the higher substrate concentration due to algae (zero-order diffusion). Organic molecules are transported through bacterial walls in an active process by species enzymes, the premeases (Billen, et al, 1980). It is important to find wether some planktonic algae posses an effective transport system, that takes up substrates at very low concentration and accumulates them inside the cells at concentrations sufficiently high for their effective introduction into the cells metabolic pathways (Hellebust, 1970). The measurement of algal diffusion is identical to that given by Wright and Hobbie, (1965;1966) and Allen, (1969;1971). The competition between bacteria and algae can be minimized by using low substrate concentration when algal populations are dense (Wright, 1973).

The dynamics of bacteria-algae dissolved organic matter interactions, is essential to solve the problem of utilization of dissolved organic matter in natural waters.

MATERIAL AND METHODS

Water samples were collected from four different aquatic systems with Ruttner sampler about 50 cm below the water surface. Samples were obtained from the middle of Koros backwater reservoir, fish ponds and about 5 km offshore the Keszthely Basin (Lake Balaton) in Hungary. Chlorophyll-a was determined by the method of Strickland and Parsons (1968).

The activity of the bacterial population was measured by the modified method of the heterotrophic potential (Wright and Hobbie, 1966). To five of the bottles, for each substrate, 100 ml aliquots of each sample were incubated in glass-stoppered bottles, for 3 hours in the dark in situ. After incubation the samples were fixed with half ml of formalin. 10 ml of subsample for each bottle was filtered through 0.2 µm Sartorius cellulose nitrate membrane filter. Each filter was then placed in a scintillation vial containing 15 ml scintillation cocktail and counted on Beckman Liquid Scintillation Counter Model L S 100 C for 10 minutes for each channel. The amino acids used were specifically labelled glycine-1-^{14}C, L-methionine (s methyl- ^{14}C), and DL-tryptophan-1-^{14}C. They were obtained from the Institute of Isotopes of the Hungarian Academy of Sciences, and had specific activities of 220.15 to 495.40 M Bq/mmole.

The observation of uptake rate of these mechanism usually showed a linear increase together with added substrate concentration (Figure 1 a and 2 a). Uptake velocity of any substrate added can be determined by the following equation :

$$v = \frac{cf(S_n + A)}{C \mu t} \qquad (1)$$

where v is the uptake velocity, µ is micro- Curies of carbon-14 added, A is the added substrate concentration (units mg carbon/m^{-3}). S_n the natural concentration of the same substrate added already found in the water sample (units mg carbon/m^{-3}), c, the radioactivity of the filtered organisms (cpm) and C the cpm from 1 micro-Curie of carbon-14, t, the incubation time in hours, while f, a factor for correction the isotope discrimination, which can be neglected (Wright and Hobbie, 1966). Also, graphical analyses from equation (2)

$$C\mu t/c = \frac{K_t + S_n}{V} + \frac{A}{V} \qquad (2)$$

where V, the maximum velocity attained when all uptake sites are saturated substrate. K_t a transport constant similar to the Michaelis-Menten constant (K_m).

By plotting Cμt/c against increasing substrate concentration S_n + A (Figures 1 b and 2 b), the slope obtained represents the diffusion constant K_d as hours. At the same time, if the natural substrate concentration (S_n) was known, (K_d h^{-1}) is used to calculate the uptake velocity (v_d) by plankton display diffusion. This uptake velocity is estimated according to equation (3)

$$v_d = K_d\ S_n \qquad (3)$$

This constant (K_d) may also be applied for estimation of the turnover time caused by diffusion uptake (T_d h^{-1}) at the natural substrate concentration and can be calculated from equation (4)

$$T_d = 1/K_d = \frac{S_n}{v_d} \qquad (4)$$

RESULTS AND DISCUSSION

The data illustrate the emerging picture of the diffusion pattern. Simple diffusion kinetics was found (Figures 1 and 2) at low and high substrate concentration during summer and winter (under ice).

In Lake Balaton (Table 1) the rate of utilization (K_d) of glycine was 19.87 x $10^{-4}h^{-1}$ and turnover time (T_d) was 503.3 hours, while the velocity of uptake (v_d) was 3.38 x 10^{-4} μg $dm^{-3}h^{-1}$ from 50 cm under ice in February, 1982. K_d of methionine during March, 1981 and February, 1982 were 123.1 x $10^{-4}h^{-1}$ and 36.65 x $10^{-4}h^{-1}$, T_d varied between 81.2 and 273.9 hours, respectively, while v_d was 17.23 and 2.2 x 10^{-4} μg $dm^{-3}h^{-1}$. In May and August, the diffusion kinetics of tryptophan were found to be faster than those methionine and glycine. K_d ranged from 40.6 to 397.3 x $10^{-1}n^{-1}$, while T_d fluctuated from 246.3 to 25.2 hours. The total algal number was 1.3 x 10^6 cell dm^{-3}, the cyanophytes as a dominant group with a total number 8.0 x 10^5 cell dm^{-3} (Padisak, 1980). The highest number was 83.4 x 10^6 cell dm^{-3} as a dominant species Aphanizomenon spp, **Synedra nana** and **Anabaena** spp (Voros, 1982).

In Koros backwater reservoir (Table 2) K_d for glycine was 40.76 and 103.49 x $10^{-4}h^{-1}$ and v_d 8.56 and 20.7 x 10^{-4} μg $dm^{-3}h^{-1}$ during July, 1981 and January, 1982, respectively. While T_d was found to be 245.3 hours in July, 1981 and 96.6 hours in January, 1982. K_d and T_d for methionine was 42.7 x $10^{-4}h^{-1}$ and 234.1 h^{-1} in October, 1981, respectively. The rate of utilization of tryptophan (K_d) fluctuated between 86.22 and 449.8 x $10^{-4}h^{-1}$ during June and September, 1981, while T_d ranged from 22.2 to 115.98 hours during September and June, 1981, respectively. It is evident

that uptake kinetics for tryptophan are higher than those for both glycine and methionine. The minimum number of algae 6.0 x 10^6 cell dm^{-3} was recorded in winter, while the maximum number of algae 15.7 x 10^6 cell dm^{-3} found in summer with a dominant groups were Chlorophyceae and diatoms (Vasas, 1980).

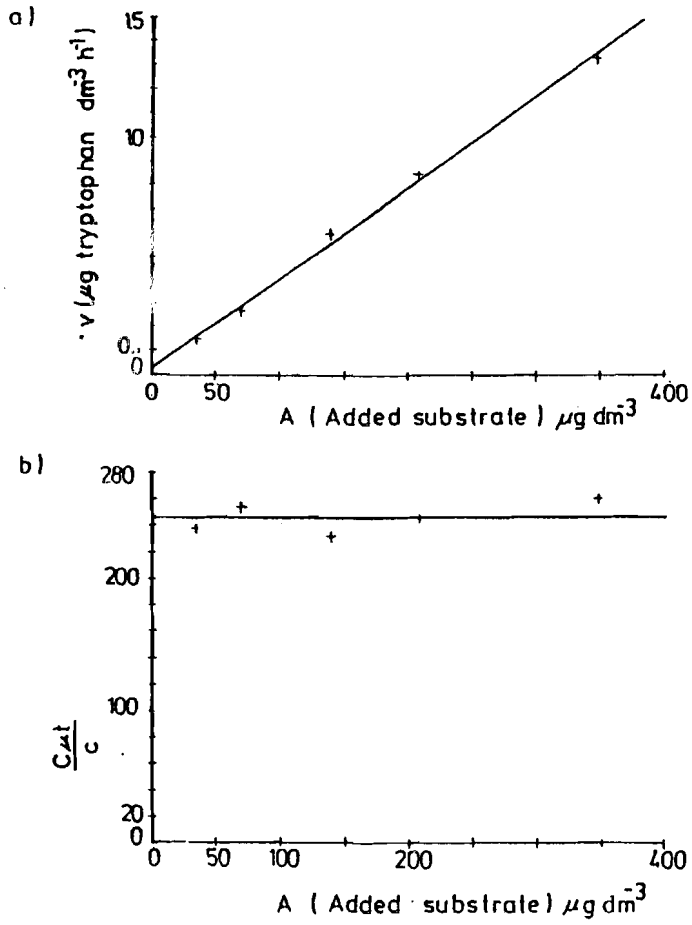

Fig. 1
Graphical analysis of the kinetics of simple diffusion
a) Plot of uptake velocity for incorporated (v) against increasing substrate concentration (A)
b) Plot of Cut/c against increasing substrate concentration. (A) Sample on 12/8/81 in Lake Balaton.

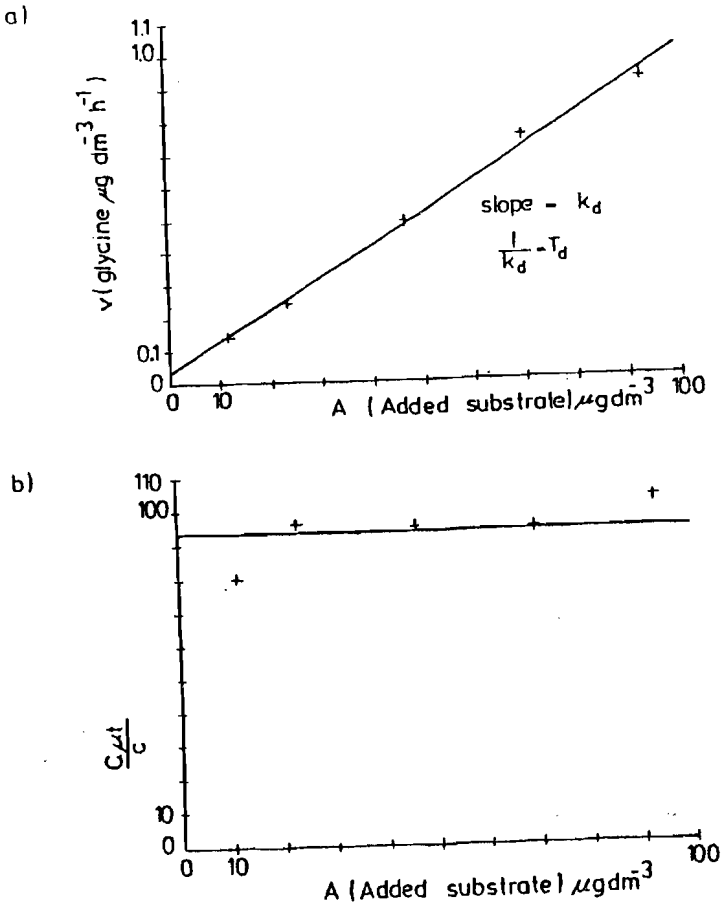

Fig. 2
Graphical analysis of the kinetics of simple diffusion
a) Plot of uptake velocity for incorporated (v) against increasing substrate concentration (A)
b) Plot of Cut/c against increasing substrate concetration (A)
Sample on 28/1/1982 in Koros backwater reservoir.

Table (1)
Diffusion kinetics for glycine, methionine and tryptophan
in Lake Balaton.

Date	4.02.1982	17.03.1981	4.02.1982	12.05.1981	3.07.1981	12.08.1981
Temperature °C	0.3	7.7	0.3	17.0	19.0	16.0
Substrate /A/	Glycine	Methionine	Methionine	Tryptophan	Tryptophan	Tryptophan
k_d h^{-1} x 10^{-4}	19.87	123.1	36.65	40.6	130.4	397.3
T_d hours	503.3	81.2	272.9	246.3	76.7	25.2
S_n µg dm^{-3}	0.17	0.14	0.06	-	-	-
V_d µg dm^{-3} h^{-1} 10^{-4}	3.38	17.23	2.20	-	-	-
Chlorophyll (a) µg dm^{-3}	-	41.71	-	18.33	17.32	56.93
A µg dm^{-3}	11 - 93	60 - 363	26 - 215	34 - 348	35 - 278	11 - 115

TABLE 2
Diffusion kinetics for glycine, methionine and tryptophan
in Koros backwater reservoir

Date	9.07.1981	28.01.1982	29.10.1981	17.06.1981	9.07.1981	7.09.1981
Temperature °C	21	0.5	7.5	25	21	17.4
Substrate	Glycine	Glycine	Methionine	Tryptophan	Tryptophan	Tryptophan
k_d h^{-1} x 10^{-4}	40.76	103.49	42.71	86.22	87.42	449.8
T_d hours	245.3	96.6	234.1	115.98	114.4	22.2
S_n µg dm^{-3}	0.21	0.20	Trace	-	-	-
V_d µg dm^{-3} h^{-1} x 10^{-4}	8.56	20.7	-	-	-	-
Chlorophyll-a µg dm^{-3}	26.97	-	-	17.1	26.97	35.59
A µg dm^{-3}	30-244	11-93	29-234	35-278	35-278	14-115

Liquid manure fertilized fish pond (Table 3) the K_d for tryptophan was 216.6×10^{-4} and $345.0 \times 10^{-4} h^{-1}$, with chlorophyll-a 17.16 and 95.86 µg dm^{-3} and the algal number was 61.66×10^6 cell dm^{-3} with **Chlorella** spp (35.402×10^6 cell dm^{-3}) as a dominant species and 62.165×10^6 cell dm^{-3} with **Scenedesmus** spp (26.594×10^6 cell dm^{-3}) as a dominant species, during August and July, 1981, respectively. Bacterial counts were 2,465 and 2.533×10^6 cell ml^{-1} during the same months. T_d was 29.0 and 46.2 hours during July and August, 1981. Shrift (1966), has demonstrated that the freshwater algae, **Chlorolla vulgaris**, take up methionine through an active transport system.

Inorganic fertilized fish pond (Table 3) the rate of utilization (K_d) for glycine was $306.98 \times 10^{-4} h^{-1}$ and T_d 32.6 hours, the chlorophyll-a was 74.21 µg dm^{-3} while the algal number 9.486×10^6 cell dm^{-3} and **Centrales** spp were the dominant species.

The bacterial count was 7.24×10^6 cell ml^{-1} in June. K_d for tryptophan was 181.9×10^{-4} and T_d was 54.98 hours, the chlorophyll-a was 98.41 µg dm^{-3} with algal number 4.404×10^6 cell dm^{-3}. **Euglena** was the dominant species and bacterial number was 7.365 cell ml^{-1} in July.

TABLE 3
Diffusion kinetics for glycine and tryptophan in liquid manure fertilized and inorganic fertilized fish ponds.

Location	Liquid manure fertilized fish pond		Inorganic fertilized fish pond	
Date	14.07.1981	17.08.1981	11.06.1981	15.07.1981
Temperature °C	25.0	25.0	27	23
Substrate	Tryptophan		glycine	tryptophan
$K_d h^{-1} \times 10^{-4}$	345	216.6	306.98	181.9
T_d hours	29	46.2	32.6	54.98
S_n µg dm^{-3}	-	-	0.78	-
V_d µg $dm^{-3} h^{-1} \times 10^{-4}$	-	-	230.23	-
Chlorophyll-a µg dm^{-3}	95.86	17.16	74.21	98.41
A µg dm^{-3}	35-278	35-278	30-244	35-278

Plankton also was responsible for the uptake beside the bacteria. Diffusion has been observed not only at high substrate concentration, but also at low substrate concentration. Wright and Burnison, (1979) observed that, diffusion occurred at very low substrate concentration in oligotrophic ocean waters. Algal communities metabolized glycine at a faster rate under ice cover than methionine. The rate of utilization (K_d) was much greater in two fish ponds than in the other aquatic bodies for glycine and tryptophan except during August in Lake Balaton and September in Koros backwater reservoir (Tables 1 and 2). T_d turnover time due to diffusion varied between 22.2 to 503.3 hours for amino acids. The results on simple diffusion for amino acids are higher than those recorded by Wright and Hobbie, (1966) for glucose and acetate. The K_d and T_d for tryptophan in all water bodies was greater than those of glycine and methionine. v_d for glycine and methionine indicates that, the diffusion uptake of plankton was lower than that of bacteria. Certainly the plankton plays a minor role in removal of free substrate from water. Wright and Hobbie (1966) mentioned that the seasonal cycle K_d was not completely known, with the lowest values late in winter and the highest values late in summer. Allen (1969) found that K_d was much greater (10^{-3} to $10^{-2}h^{-1}$) in Lake Lotsjon than that measured in other water bodies (K_d 10^{-5} to $10^{-4}h^{-1}$) for glucose and acetate. Relatively longer turnover time (T_d) of algal diffusion for glycine and methionine was measured (Td 503.3 and 272.9 hours) under ice period in Lake Balaton, while the turnover time for tryptophan was relatively shorter. These values are comparable to that of Allen (1969) who foun a T_d for glucose and acetate varying between 150-500 hours. He also stated that bacteria were much more responsible for the uptake than the algae. Bacterial uptake was quantitative more important than algal uptake by results of different filtrations in lake water (Wright and Hobbie, 1966).

The relation between the diffusion uptake and the bacteria in such water bodies, may be attributed to inactive state or the low bacterial numbers or that very small algae might have transport systems. Allen (1969) mentioned that diffusion of organic compounds was greatest under ice cover. His results agree with our data. Wright and Hobbie (1966) have suggested that nannopkanktonic algae with surface area/volume ratios approximating the same of the bacteria and may be competitive with the bacteria for organic substrates by utilizing active transport mechanisms.

Taking into account these results, it can be concluded that, the work presented in this paper gives supplimentary informations about the algal uptake. Out of 150 experiments on heterotrophic uptake, it was found that 16 experiments did not show proper response to Michaelis-Menten kinetics. The response of algal communities to simple diffusion kinetics, indicates that they are taking part in the removal of dissolved organic matter from natural water. However, it should be mentioned that bacteria is the main consumer of particulate organic matter. Our studies show that the entire diffusion uptake do not mainly depend on the substrare concentration, but, the environmental and ecological conditions (light,

density of population, ...) may also interfere. The blooming of algae, and the major of microalgae have transport system acting like that of bacteria. Under extreme conditions (eg. in the presence of ice cover), to preserve the population, autotrophic algae are able to extract simple organic molecules from the water column. Allen (1971) answering the question : how important are the algae?, stated that if active transport mechanisms are found for a large number of small algae, they are important in the cycling dynamics of dissolved organic materials in aquatic ecosystems. Hellebust and Guillard (1967) showed that the marine diatom **Melosira nummuloids** is highly selective in its ability to take up organic substrates, it does not take up sugars, or organic acids to a significant extent, but readily takes up any amino acid present in the medium at relatively low concentration.

REFERENCES

Allen, H.L. 1969. Chemo-organotrophic utilization of dissolved organic compound by planktic algae and bacteria in a pond. Int. Revue ges. Hydrobiol., 54: 1-33

Allen, H.L. 1971. Dissolved organic carbon utilization in size-fractionated algal and bacterial communities. Int. Revue ges. Hydrobiol., 56: 731749.

Billen, G.; C. Joiris; J. Wijnant and G. Gillain 1980. Concentration and microbiological utilization of small organic molecules in the Scheldt Estuary, the Belgian Coastal Zone of the North Sea, the English Channel. Estuar. Coast. Mar. Sci., 11: 279-294.

Figg. G.E. 1966. The extracellular products of algae. Oceanogr. Mar. Biol. Ann. Rev., 4: 192-212.

Hellebust, J.E. 1970. The uptake and utilization of organic substances by marine phytoplankton. pp. 225-256 In: Hood, D.W. (ed.), Symposium on organic matter in natural waters. Institute of Marine Science, University of Alaska, USA.

Hellebust, J.A. and R.R. Guillard 1967. Uptake specificity for organic substances by the marine diatom **Melosira nummuloides.** J. Phycol., 3: 132-136.

Padisak, J. 1980. Short-term studies on the phytoplankton of Lake Balaton in the summers of 1976, 1977, and 1978. Acta Botan. Acad. Sci. Hung., 26: 397-416.416.

Parsons, T.R. and J.D.H. Strickland 1962. On the production of particulate organic carbon by heterotrophic processes in sea water. Deep-Sea Res., 8: 211-222.

Shrift, A. 1966. Methionine transport in **Chlorella vulgaris.** Plan Physiol., 41: 405-410.

Strickland, J.D. and T.R. Parsons 1968. A practical handbook of water analysis. Bull. Fish. Res. Bd. Can., 167: 311 p.

Vasas, F. 1980. Quantitative studies on the phytoplankton in the Backwater of Szarvas. Aqua. Hung. 2: 71-87.

Voros, L. 1982. Quantitative and structural changes of phytoplankton in Lake Balaton between 1965-1978. Aqua. Hung. 3: 137-144.

Wood, E.J.F. 1959. Some aspects of marine microbiology. J. Mar. Biol. Assoc. India, 1: 26-32.

Wright, R.T. 1973. Some difficulties in using C-14 organic solutes to measure heterotrophic bacterial activity, pp. 199-217. In: Stevenson, L.H. and R.R. Colwell, (eds.), Estuarine Microbiol. Ecology, South Carolina Press, Raleigh, USA.

Wright, R.T. and B.K. Burnison 1979. Heterotrophic activity measured with radiolabelled organic substances. Native Aquatic Bacteria : Environment, Activity, and Ecology ASTM STP 695 p., Costerton, J.W. and R.R. Colwell, (Eds.), Amer. Soc. for Testing and Materials. pp. 140-155.

Wright, R.T. and J.E. Hobbie 1965. Competition between bacteria and algae for organic solutes. Mem. Ist. Ital. Idrobiol. 18 suppl., 175-185.

Wright, R.T. and J.E. Hobbie 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology, 47: 447-464.