APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1983, p. 1761–1766
0099-2240/83/061761-06$02.00/0

Vol. 45, No. 6

Microbial Activity at the Sediment-Water Interface in Halifax Harbor, Canada

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Received 29 November 1982/Accepted 14 March 1983

The sediment-water interface in Halifax Harbor supports a microbial population of 6.95×10^9 cells per g (dry weight). As determined by the standard technique of suspending subsamples in filtered seawater, the uptake of added glutamic acid by this population is 113.5 ng g (dry weight)$^{-1}$ h^{-1}. An alternate technique was developed to measure the heterotrophic activity of the interface over longer periods of time, using undisturbed cores with the sediment-water interface intact. Under these conditions, the microbes in the water column and the interface increased exponentially in number, with mean doubling times of 9.6 and 4.5 days, respectively. The uptake of glutamic acid by the microbial population of the interface was determined to be 12.7 ng g (dry weight)$^{-1}$ h^{-1}, almost an order of magnitude less than the uptake determined by the previous method. This indicates that substrate diffusion and competition for substrate by the microbes in the water column are important factors when considering the heterotrophic activity of the sediment microbial population. After 48 h of incubation, uptake and respiration ceased, probably due to the exhaustion of labeled substrate. Additional substrate added after 48 h of incubation was taken up at a rate similar to that measured after the first addition. It appears that the microbial population of the interface is able to respond quickly and repeatedly to relatively large nutrient additions. After 10 days of incubation, the number of "viable" cells as determined by autoradiography was much smaller than the increase in numbers as determined by direct counts. Apparently a large part of the viable population is unaffected by nutrient addition.

A previous study (13) has identified the sediment-water interface of a coastal marine bay as an area of intense microbial activity. Because of this activity, the interface must be considered as an important part of the sediment-water ecosystem. Unfortunately, few studies have investigated the microbiology of the marine sediment-water interface (1, 2, 13), and, to my knowledge, only one such study has addressed the problem directly (13). Likewise, the freshwater sediment-water interface has also not been studied. Oláh (17) examined the sediment-water interface of shallow lakes and found generally higher viable counts at the sediment surface than in the water column or deeper in the sediment but presented no data on microbial activity.

To understand the microbial processes at the sediment-water interface and to determine what factors influence these processes, the present study was initiated. Specifically, it was the purpose of this study to characterize the microbiology of the interface and to examine a method to measure the heterotrophic activity of the microbial population of the interface. Since the interface is, by definition, composed of both sediment and water components, I decided to study the interface by keeping all components of the sediment-water system intact. By using techniques that use entire core samples, I attempted to collect data that would be more representative of the natural system than those collected by using a disected-system approach.

MATERIALS AND METHODS

Sampling site and sample collection. The sampling site used in this study was the area of Halifax Harbor known as Eastern Passage, which was also the site of previous studies (8, 9, 13, 14, 16). It is characterized as a 22-m water column overlying a muddy bottom. All samples were collected by hand by scuba divers. Undisturbed sediment-water interface samples were collected and subsampled as previously described (13). For long-term incubation of the sediment-water system, excess water was carefully removed from the core tube so that 300 ml of bottom water remained on top of the sediment core. The core was then capped with a rubber stopper connected by tubing to a gassing manifold. A hydrated air-nitrogen mixture flushed the head space of the core (approximately 300 ml) at a rate of 200 ml/min. The air-nitrogen mixture was adjusted to maintain the overlying water at in situ O_2 concentra-

tions (9). The entire assembly was contained in a low-temperature incubator (Psychrotherm; New Brunswick Scientific) at the in situ temperature of 2°C. Incubations were carried out in the dark, as measurements of light intensity at the sampling site indicated that <0.1% of the light measured at the water surface reached the sediment surface.

Standard heterotrophic activity measurements were made on the interface as previously described (13, 14), using [*U*-^{14}C]glutamic acid (specific activity, 267 mCi/mmol). Heterotrophic activity measurements conducted with undisturbed cores were made by adding, [*U*-^{14}C]glutamic acid (specific activity, 267 mCi/mmol) [*U*-^{14}C]glucose (specific activity, 301 mCi/mmol), or [*U*-^{14}C]aspartic acid (specific activity, 225 mCi/mmol).

Additions were made so that the final concentration of labeled substrate in the overlying water was equal to that used for the described heterotrophic activity measurement, 0.01 μCi/ml. Core tubes containing 300 ml of bottom water only were used to measure the heterotrophic activity of the water without sediment, and cores (with and without sediment) killed with 0.2% (final concentration) Formalin served as controls. At various times samples were taken and processed as follows: 10-ml water samples were pipetted from the cores without sediment and carefully removed with a syringe from the cores containing sediment. Twenty-milliliter samples of interface slurry were aspirated from one-fourth of the sediment surface with a 50-ml syringe fitted with a Teflon catheter as previously described (13). Five-milliliter aliquots of the water samples and 5-ml portions of the interface slurries were placed into 50-ml serum bottles. The bottles were capped with stoppers fitted with cups containing fluted filter paper, and the reaction was immediately stopped with the injection of 1 ml of 3 N H_2SO_4. The samples were then processed in the standard manner as described previously (13). The remaining portion of the interface slurry was used to determine the dry weight of the sediment sampled.

For microautoradiography, L-[3,4-^{3}H]glutamic acid (specific activity, 44 Ci/mmol) was added to the bottom water in the same molar concentration as the [^{14}C]glutamic acid added for the heterotrophic activity measurement as described above. [*methyl*-^{3}H]thymidine (specific activity, 77.2 Ci/mmol) was added at a final concentration of 5 nM. Autoradiograms were prepared according to the method of Meyer-Reil (10) as modified by Novitsky (13). All autoradiograms were exposed for 21 days. Total cell counts were determined by acridine orange epifluorescence microscopy (13).

Aspiration of the interface from the entire area (52.8 cm^2) of individual sediment cores produced an average value of 0.75 g (dry weight) of interface per core. Using this measurement and a volume of 300 ml for the bottom water, the amount of substrate uptake for the entire core was calculated. Values were determined for substrate respired as CO_2 or incorporated into macromolecules in the water column and interface slurry. Uptake rates were calculated from the slope of the initial linear portion of the uptake plots.

TABLE 1. Acridine orange direct counts of interface samples taken at Eastern Passage

Core	Cells per g (dry wt) ± 95% confidence interval
1	$3.13 \times 10^9 \pm 7.71 \times 10^8$
2	$7.09 \times 10^9 \pm 1.37 \times 10^9$
3	$5.68 \times 10^9 \pm 1.58 \times 10^9$
4	$9.87 \times 10^9 \pm 1.57 \times 10^9$
5	$5.76 \times 10^9 \pm 1.14 \times 10^9$
6A	$1.10 \times 10^{10} \pm 1.21 \times 10^9$
6B	$7.25 \times 10^9 \pm 1.87 \times 10^9$
6C	$5.88 \times 10^9 \pm 1.11 \times 10^9$
6D	$6.95 \times 10^9 \pm 1.17 \times 10^9$
7A	$7.30 \times 10^9 \pm 9.45 \times 10^8$
7B	$5.62 \times 10^9 \pm 1.48 \times 10^9$
7C	$9.70 \times 10^9 \pm 1.18 \times 10^9$
7D	$5.12 \times 10^9 \pm 1.38 \times 10^9$
$\bar{x}$	$6.95 \times 10^9 \pm 1.31 \times 10^9$

RESULTS

The sediment-water interface at the Eastern Passage sampling site of Halifax Harbor appears to be relatively homogeneous with respect to the microbial population and its activity. Direct counts of the complete interface from five cores and from four subsamples of each of two cores (13 total counts) are presented in Table 1. The average number of cells per gram (dry weight) was determined to be $6.95 \times 10^9 \pm 1.31 \times 10^9$ (mean ± 95% confidence interval). The heterotrophic activity measured for four subsamples from each of four cores (16 total samples) is shown in Table 2. The average heterotrophic uptake of glutamic acid, using the standard technique, was determined to be 113.5 ± 13.8 ng g (dry weight)$^{-1}$ h^{-1} (mean ± 95% confidence interval). I tested the null hypothesis that there were no significant differences in heterotropic activity between cores. I could not reject the hypothesis based on one-way analysis of variance ($F_{(3,12)} = 2.12$; $P > 0.05$).

The effect of long-term (up to 10 days) storage of unamended cores on the microbial population of the interface and overlying water is shown in Fig. 1. The cell populations for both the water and interface increased logarithmically with mean doubling times of 9.6 and 4.5 days, respectively. With added glutamic acid, the number of active cells in the interface population as determined by autoradiography also increased logarithmically, with a mean doubling time of 9.4 days (Fig. 1). With added thymidine, the number of active cells actually decreased as shown in Fig. 1. The percentage of the population that was active with respect to glutamate did not exceed 10.5%, whereas the percentage of the population active with respect to thymidine did not exceed 9.5%. Even in the overlying water, the percentage of the population active with respect to glutamic acid or thymidine did not exceed 12 or 7%, respectively. One core was amended with tritiated thymidine at time zero

TABLE 2. Uptake of glutamic acid by the microbial population of interface samples taken at Eastern Passage[a]

Core	Uptake of glutamic acid (ng g [dry wt]$^{-1}$ h^{-1})					±95% Confidence interval	SD	Coefficient of variation (%)
	Subscore							
	A	B	C	D	$\bar{x}$			
1	117.3	121.4	80.5	161.1	120.1	52.3	32.9	27.4
2	87.2	91.4	112.0	154.7	111.3	49.0	30.8	27.7
3	95.4	96.7	75.3	96.8	91.1	16.7	10.5	11.5
4	124.3	139.3	126.5	136.0	131.5	11.6	7.3	5.6
All samples					113.5	13.8	25.7	22.8

[a] Heterotrophic activity was measured by the standard method.

and again after 48 h. Autoradiography of the microbial population of the interface after the second addition of substrate (48 to 96 h) showed no further increase in the number or the percentage of active cells. Direct counts for water samples stored in the presence of sediment did not increase and actually had a slight negative slope (data not shown). Standard 2-h heterotrophic activity measurements of interface subsamples from stored cores at various times up to 10 days showed that the heterotrophic activity slowly increased linearly at a rate of 6.3 ng of glutamic acid uptake g (dry weight)$^{-1}$ h^{-1} day^{-1} (data not shown).

The uptake of glutamic acid over time by the microbial population of the interface is shown in Fig. 2. Sampling as close as 4 h after substrate addition did not reveal any significant lag period in substrate uptake, which proceeded linearly for at least 36 h. After 48 h, uptake essentially stopped. After 48 h, small decreases were noted in the amount of labeled CO_2 and macromolecules present. Similar results were also noted when either glucose or aspartic acid was used as substrate (data not shown). For all three substrates, uptake rates were calculated as the slope of the initial linear portion of the uptake curve. By calculating the total uptake rate (CO_2 plus acid-precipitable material in the water column and interface) and subtracting the rate determined for water only (no sediment present), the uptake due solely to the microbial population of the interface could be determined for each of the three substrates (Table 3).

In the final experiments, unlabeled glutamatic acid was added to cores at time zero to a concentration equal to that used when radioactive substrate was used. After 48 h, radioactive substrate was added and uptake was monitored. The rate of uptake was only slightly greater (25.32 versus 21.34 ng core^{-1} h^{-1}) than in interface samples not previously exposed to substrate (Fig. 3). The major difference was the greater amount of CO_2 produced by the population that received two portions of substrate. After the second addition of substrate, neither the total number of cells, the number of active cells, nor the percentage of active cells increased significantly for either the interface or the overlying water microbial population.

DISCUSSION

The true sediment-water interface, the actual boundary between the sediment and the water column, can only be considered theoretically. For practical purposes, the sediment-water interface has been given a functional definition for this study. In fact, the sampling procedure largely defines what is measured and termed as

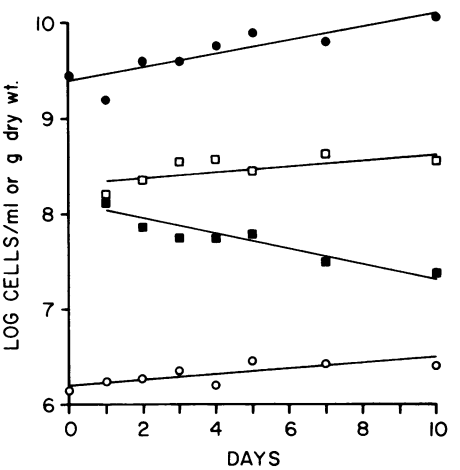

FIG. 1. Acridine orange total, and microautoradiography active, direct counts during long-term incubation of sediment cores. Symbols: Acridine orange total counts of the water column (○) and of the interface (●); active cells as determined by autoradiography of the interface, with glutamic acid as the substrate (□), and with thymidine as the substrate (■). The lines drawn represent the straight line of best fit, using a least-squares linear regression analysis. For total cells in the interface: $y = 0.07x + 9.42$; $r = 0.85$. For total cells in the water column: $y = 0.03x + 6.19$; $r = 0.82$. For glutamate-active cells in the interface: $y = 0.03x + 8.32$; $r = 0.70$. For thymidine-active cells in the interface: $y = -0.08x + 8.13$; $r = -0.94$.

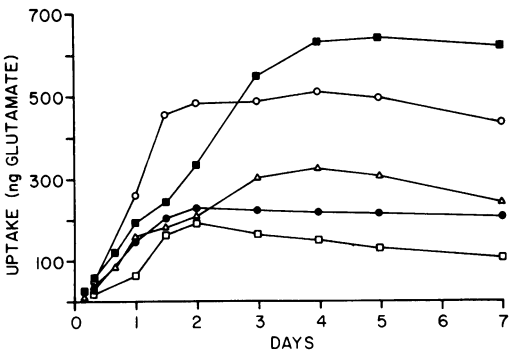

FIG. 2. Long-term glutamic acid uptake experiments used to determine the uptake rate of the microbial population of the interface. Symbols: Uptake of glutamic acid by a sediment-water core—respired CO_2 (○), acid-precipitable macromolecules in the water column (●), and acid-precipitable macromolecules in the interface (□); uptake of glutamic acid by a water only sample—respired CO_2 (■), and acid-precipitable macromolecules (△).

"interface." For the purposes of this discussion, the term "sediment-water interface" will indicate the top layer of sediment (<1 mm) as sampled in this and a previous study (13). Unfortunately, others (18) have used the terms sediment and sediment-water interface interchangeably. As shown in a previous study (13), the interface is quite distinct from the sediment immediately below.

From the direct counts and heterotrophic activity measurements (Tables 1 and 2) of interface samples taken at different times but within the same general sampling area, it appears that the microbial population of the interface is rather homogeneous. This is important in long-term experiments in which several cores are used due to the limited amount of material that can be removed from any one core. The largest inter- and intracore differences probably stem from the sampling technique. It is very difficult to control the collection of the interface material, and undoubtedly some underlying sediment and overlying water is also collected with the interface. My bacterial abundance figures for the interface are in general agreement with those of Dale (3), who found 1.17×10^8 to 9.97×10^9 cells per g (dry weight) in an intertidal basin in Nova Scotia. Although he sampled the sediment rather than the interface, his are the only other abundance figures available for Nova Scotia coastal sediment. Although the bacterial numbers in the interface are higher than the sediment immediately below (13), even higher numbers have been found (1.3×10^{11} cells per g, dry weight) in some marine muds (12).

This technique of incubating whole cores is similar in concept to the "bell jar" type of experiments (for examples, see references 1 and 2) in which some type of enclosure is placed over an area of sediment. Ports in the enclosure allow water samples to be taken at various times. The technique described in this study has the advantages that the water or sediment can easily be sampled and that in situ oxygen concentrations can be maintained. Totally enclosed, the bacterial population of the sediment can easily and quickly deplete the oxygen supply in the overlying water (2). In addition to maintaining the sediment and interface undisturbed, the present technique considers the sediment and water as an integrated system. It also makes no distinction among the various heterotrophic groups; activity is measured as overall heterotrophic activity. A major disadvantage of the technique is the containment of the sediment and water for long periods of time. Even though there were increases in cell numbers and activity over time, the increases were not significant over the period of time (0 to 48 h) during which the rate measurements were made. Although experiments can be and were carried out for periods of up to 10 days, it is probably best to keep the incubation as short as possible.

During long-term incubation of the sediment cores, the microbial population of the interface increased logarithmically, with a mean doubling time of 4.5 days (Fig. 1). The population of the overlying water also increased, but at a slower rate (mean doubling time, 9.6 days; Fig. 1).

TABLE 3. Uptake rates for microbial populations of the sediment-water core samples collected at the Eastern Passage sampling site

Substrate	Total uptake[a]			Uptake for water only			Uptake rate for interface only[b]	
	Rate (ng $core^{-1}$ h^{-1})	Time (h)	r	Rate (ng [300 ml]$^{-1}$ h^{-1})	Time (h)	r	ng $core^{-1}$ h^{-1}	ng g (dry wt)$^{-1}$ h^{-1}
Glutamic acid	21.3	0–36	0.98	11.7	0–72	0.99	9.6	12.8
Glucose	19.6	0–48	0.97	2.4	0–168	0.99	17.2	22.9
Aspartic acid	12.7	0–48	0.99	5.5	0–48	0.97	7.2	9.6

[a] Total uptake includes substrate converted to CO_2 and acid-precipitable macromolecules.
[b] Calculated as the difference between the total uptake of the interface plus water and the uptake of water samples only.

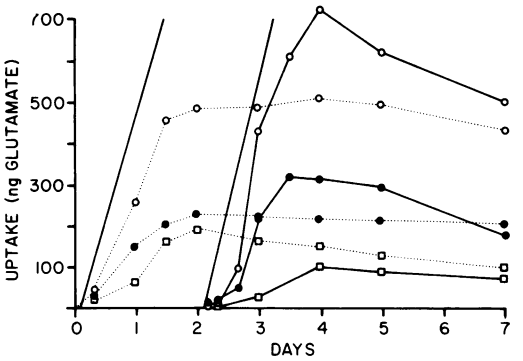

FIG. 3. Uptake of glutamic acid by the microbial population of a sediment core after previous exposure to glutamate. Unlabeled glutamic acid was added to the sediment core at time zero and labeled glutamic acid was added after 48 h. Symbols: Respired CO_2 (○); acid-precipitable macromolecules in the water column (●); acid-precipitable macromolecules in the interface (□). Data from a previous experiment showing the uptake of glutamate from time zero (dotted lines) has been reprinted from Fig. 2 for comparison. Solid lines without indicated points represent the total uptake rate for each experiment. For substrate additions at time zero: $y = 21.3x - 48.9$; $r = 0.98$. For substrate additions after 48 h: $y = 25.3x - 48.7$; $r = 0.98$.

Although it is interesting to speculate, these figures probably have no relation to bacterial growth rates in vivo. In addition, the calculated mean doubling time is an average figure; undoubtedly portions of the population are both slower and faster growing. In an attempt to arrive at a growth rate closer to the actual in vivo rate, microautoradiography was used. With glutamic acid as a substrate, the number of active cells increased with a mean doubling time of 9.4 days (Fig. 1). The addition of substrate did not affect the increases in cell numbers. Even when a second portion of substrate was added (Fig. 3) no significant increase in cell numbers was noted. With thymidine as a substrate, the number of active cells actually decreased (Fig. 1). With so few active cells measured, either the autoradiography technique is faulty or the cells are not taking up the substrates. The validity of the autoradiography technique has been discussed previously (5, 13). In a previous study (13) I showed that, in general, a greater percentage of the sediment and water column microbial population took up glutamate than took up thymidine. Fuhrman and Azam (5) claim that all of the active cells take up thymidine. Since thymidine is incorporated into DNA, it is possible that nondividing cells would metabolize glutamate, but not thymidine. In the present study, however, direct counts clearly show an increase in cell numbers in the interface from 3.13×10^9 to 1.07×10^{10} cells per g (dry weight) over the 10-day incubation period. Clearly, at least 7.57×10^9 cells per g are active, viable, and dividing. The maximum total number of active cells as determined by autoradiography (4.19×10^8 glutamate-active cells per g plus 1.5×10^8 thymidine-active cells per g) represents only 7.5% of the increase in cell numbers. It appears from these data that the vast majority of the actively dividing cells are not taking up either glutamate or thymidine. In the event that the labeled substrate was being initially respired and hence not appearing in cellular material, additional portions were added after 48 h, but no increase in active cells was noted for either substrate. The possibility of respiration cannot be ruled out. The decrease in the number of thymidine-active cells (Fig. 1) indicates the loss of label from the cells, possibly due to respiration. Perhaps DNA is not a stable component in marine bacterial cells, especially in those under nutrient limitation as shown by Novitsky and Morita (15). If radiolabeled substrates are not being taken up or are being respired by growing cells, their use in estimating microbial growth rates would underestimate the true microbial production. Although Fuhrman and Azam (5) presented an empirical relationship between thymidine incorporation and bacterioplankton production, Ducklow et al. (4) found their thymidine-based estimates of bacterial growth rates significantly lower than the estimates based on changes in cell numbers.

The uptake of glutamate, glucose, or aspartate by the microbial population of the interface-water system was similar: linear uptake for approximately 48 h followed by a decrease in the amount of $^{14}CO_2$ retained in the water and the macromolecules retained in the cells. The reduction in measured CO_2 probably represents diffusion of the $^{14}CO_2$ into the sediment and exchange with the atmosphere above the core, which was continuously flushed with an air-nitrogen mixture. The loss of macromolecules from the cells was unexpected. Apparently a portion of the newly synthesized macromolecular cellular components is hydrolyzed and excreted or respired. No additional uptake is noted after 2 days of incubation (see Fig. 2), probably due to the total exhaustion of the substrate. When another portion of substrate is added (Fig. 3), uptake is essentially the same as that observed after the first addition. Apparently the microbial population is not significantly changed by the first addition. The microbial population also appears to respond quickly to subsequent nutrient addition. As I have pointed out in a previous study (13), without knowing the natural substrate concentrations, the actual heterotrophic uptake rates cannot be calculated. However, since the amount of nutrient added is equal to

the amount used in the measurement of heterotrophic activity by the single substrate concentration technique of Griffiths et al. (6) used in this and other studies (13, 14), the rates obtained here should correspond to previous measurements. However, the glutamate uptake rate calculated for the interface with the whole-core technique (12.8 ng g [dry weight]$^{-1}$ h^{-1}; Table 3) is almost an order of magnitude less than that measured (113.5 ng g [dry weight]$^{-1}$ h^{-1}; Table 2) with the previous technique, using shaken subsamples diluted in filtered seawater. Shaking a diluted sample of sediment is obviously quite different from in situ conditions. Probably the largest difference is the elimination of diffusion of the substrate as a controlling factor. This study graphically demonstrates the importance of substrate diffusion into sediments as a controlling factor of microbial activity. Also, since the substrate is added to the water column, competition for that substrate by the bacterial population in the water column presents additional demands on the available substrate for the sediment microorganisms. Hall et al. (7) examined lake sediment and found the uptake rates for undisturbed sediment cores to be an order of magnitude lower than those measured with shaken subsamples. Meyer-Reil (11) found uptake rates for undisturbed marine sediment much lower than other reported values; unfortunately, no data are reported on the effect of diffusion of substrate into the sediment as substrate was physically forced into the sediment by applying a vacuum to the sediment column.

Since the water column is interacting with the sediment in these experiments, the percentage of substrate that is respired is a function of both the sediment and the water column populations. Although this may seem desirable, the actual figure calculated is biased by the amount of water overlying the sediment. Hence, the more water, the closer the percent respired will be to the percent respired of the water column population. For this reason, these figures can be used only for relative comparison. Using figures for 52.8 cm^2 of sediment and 300 ml of water, the percentages respired were 28, 59, and 64% for glucose, glutamic acid, and aspartic acid, respectively. The question of the importance of the interface relative to the entire ecosystem also is constrained by the amount of water overlying the sediment. Assuming the average water depth to be 22 m and with an average glutamate uptake activity of 12 pg ml^{-1} h^{-1} (13), total uptake for the water column over 52.8 cm^2 of sediment is 1.4 μg/h. The uptake calculated in this study for 52.8 cm^2 of interface is only 9.6 ng/h. Clearly, the interface is only part of the activity of the entire system, but on a weight or volume basis it represents the most active microbial habitat in this ecosystem. The large and active population of the interface may be needed for, and play a large role in, recycling the vast array of relatively refractory compounds deposited on sediments in the natural environment.

ACKNOWLEDGMENTS

For expert diving and field services I acknowledge the Richard W. Welsford Research Group, Ltd., especially Rick Welsford and John Stairs.

This research was funded by grant A-6548 from the National Research Council of Canada.

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