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# THE UPTAKE OF ORGANIC COMPOUNDS BY HETEROTROPHIC BACTERIA IN RELATION TO GROWTH RATE*.

Antonie B.J. SEPERS
Delta Institute for Hydrobiological Research Vierstraat 28,
4401 EA YERSEKE, THE NETHERLANDS

## RESUME

## L'UPTAKE DE COMPOSES ORGANIQUES PAR UNE BACTERIE HETEROTROPHE EN RELATION AVEC LE TAUX DE CROISSANCE.

La souche bactérienne HIS 42 a été cultivée dans un chemostat histidine-limité à des taux de croissance de 0,01, 0,05 et 0,1 $h^{-1}$. Sur des échantillons de cette culture l'uptake de différents composés organiques a été mesuré avec des substrats $^{14}C$. La souche bactérienne HIS 53 a été cultivée dans un chemostat aspartate-limité a des taux de croissance de 0,003 et 0,001 $h^{-1}$. Sur des échantillons de cette culture l'uptake de différents acides aminés a été déterminé par la mesure du taux de consommation d'oxygène à saturation en substrat.

Dans ces conditions de culture l'utilisation d'un certain nombre de composés organiques pourrait être démontrée, indiquant que l'uptake simultané de plusieurs composés organiques dans des conditions naturelles est très vraisemblable. De plus, ces expériences on démontré que l'uptake pour un certain substrat augmente avec l'accroissement du taux de croissance spécifique, dans la mesure où le substrat limitant la croissance est concerné. Cette augmentation représente le comportement adaptatif des organismes au taux de croissance supérieur. Pour les autres substrats une relation différente entre l'uptake et le taux de croissance ne peut pas être évaluée.

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## INTRODUCTION

Heterotrophic bacteria are able to utilize many organic compounds as the sole carbon and energy source (Sepers, 1981; Berland *et al.*, 1972). This can be considered as an indication for the simultaneous uptake of several organic compounds by heterotrophic bacteria in the natural environment.

Another indication for the simultaneous uptake of organic compounds comes from physiological experiments, dealing with the cell composition of micro-organisms in relation to growth rate. It is a generally observed phenomenon that the protein content of the cell is nearly constant over a wide range of growth rates, whereas the RNA content sharply increases with increasing growth rate (Herbert, 1961; Harder and Veldkamp, 1967). The increase in the RNA level represents an increase in the fraction of ribosomal ribonucleic acid, which is necessary in order to realize the higher growth rates. Simultaneously an increase of the protein content of the cells would be expected, in accordance with the presence of more ribosomes in the cells. The absence of this expected increase can be explained by the next supposition. Considering the nearly constant level of the total protein content of the cell, at low growth rates with lower concentrations of ribosomal protein, another protein fraction predominates. The decrease of this protein fraction and the increase of the ribosomal protein fraction level off with increasing growth rates, resulting in a nearly constant protein level of the cell.

The indicated protein fraction may be made up of enzymes involved in the uptake and subsequent metabolism of a variety of organic compounds. The higher concentration of these uptake systems at low growth rates has ecological importance. In natural aquatic environments, the specific growth rates of micro-organisms are very low, as is the case with the concentration of the organic compounds permitting heterotrophic growth. The increase of the protein fraction in question at the lower growth rates could be the result of an increase of the concentration of enzymes already present, and of the appearance of new enzymes which permit the uptake and assimilation of a greater variety of organic substrates. If the above mentioned hypothesis is correct, then bacteria are able to utilize simultaneously more organic substrates at the lower growth rates, which normally occur in the natural environment, than at the higher growth rates. The effect of the simultaneous uptake of a number of organic compounds is a more efficient utilization of the growth limiting nutrients with the ultimate result that the growth rate increases.

Furthermore a theoretical study of Krambeck (1979) dealing with the applicability of the Michaelis-Menten kinetics in microbial ecology, showed that in enzyme networks the saturation constant K is a function of all maximum reaction rates of the intermediary enzymes. A consequence of this phenomenon is that organisms can regulate their saturation constants by adapting their relative enzyme concentrations.

Referring to the above mentioned theoretical considerations, an experimental study was started of the relation between the growth rate and the uptake of a range of organic compounds by heterotrphic bacteria. In this paper some pilot experiments will be presented, focused on the concentration of the enzyme systems, necessary for the transport of organic compounds and the subsequent utilization in respiratory and biosynthetic pathways.

## MATERIALS AND METHODS

The experiments were done with bacterial strains HIS 42 and HIS 53, which were isolated from the freshwater Haringvliet basin in The Netherlands by enrichment in a chemostat culture at a dilution rate of 0.05 $h^{-1}$ and with histidine as the growth limiting nutrient (Sepers, 1981). The maximum specific growth rate ($\mu_{max}$) of HIS 42 for growth on histidine is 0.20 $h^{-1}$; the $\mu_{max}$ of HIS 53 for growth on aspartate is 0.32 $h^{-1}$.

Bacterial strain HIS 42 was grown in a histidine limited chemostat at specific growth rates of 0.01, 0.05 and 0.1$h^{-1}$; at the applied growth conditions the biomass of the HIS 42 culture was nearly constant (circa 60 mg/l protein). Samples taken from the chemostat, were used to determine the uptake kinetics for histidine, glycine, leucine, aspartate, a mixture of amino acids, glucose and acetate. The methodology for the determination of these uptake kinetics was principally that of Hobbie and Crawford (1969) with some minor modifications as described by Sepers and van Es (1979). The maximum uptake

rate, as measured with this technique, is proportional to the concentration of the uptake systems for the considered substrate.

Bacterial strain HIS 53 was grown in a chemostat with aspartate as the growth limiting substrate at the specific growth rates of 0.003 and 0.01 $h^{-1}$; the biomass of the culture was about 40 mg/l protein and did not vary with the growth rate. Samples taken from these chemostat cultures were used to determine the uptake kinetics for several amino acids by measurement of the oxygen consumption rate with a Biological Oxygen Monitor (Yellow Spring Insutruments, Model 53 SA). The oxygen consumption rate measured at saturating substrate concentrations is proportional to the concentration of the uptake system of the considered substrate.

## RESULTS

In the first series of experiments bacterial strain HIS 42 was grown in a histidine limited chemostat at specific growth rates of 0.01, 0.05 and 0.1 $h^{-1}$. As is shown in Table 1, an increase of the growth rate is accompanied by an increase of the maximum uptake rate, as far as the growth limiting substrate, in this case histidine, is concerned. The maximum uptake rate of the other substrates is nearly independent of the growth rate.

For a next series of experiments bacterial strain HIS 53 was grown in an aspartate limited chemostat at the very low specific growth rates of 0.003 and 0.01 $h^{-1}$. Some indicative results are presented in Table 2. Similar to the results obtained with HIS 42, an increase of the growth rate resulted in an increase of the maximum uptake rate of the growth limiting substrate, aspartate. The maximum uptake rates of glutamate and asparagine were greater at the higher growth rate and varied nearly proportional with the maximum uptake rate determined for aspartate. For the other substrates, a clear relation between the maximum uptake rate and the growth rate could not be demonstrated.

## DISCUSSION

Summarizing the results of the presented experiments, it is quite clear that over a wide range of growth rates the uptake capacity for several organic compounds could be demonstrated (Table 1 and 2), indicating that a simultaneous uptake of several organic compounds under natural conditions is very likely.

Moreover, the experiments showed that the uptake capacity increases with an increase of the specific growth rate, as far as the growth limiting substrate is concerned. This increase is explained by the adjustment of the physiology of the organism to the higher growth rates at cultivation in a chemostat culture. For a discussion about the difference between the actual uptake rate of histidine in the HIS 42 culture and the maximum uptake rate measured with $^{14}C$-labelled histidine (Table 1), the reader is referred to Sepers and van Es (1979).

The maximum uptake rate of glutamate and asparagine of HIS 53, grown at an aspartate limitation, increased with growth rate and varied nearly proportional with the maximum uptake rate of aspartate (Table 2). These results may be explained by the direct involvement of the substrate in the citric acid cycle after deamination. For the other substrates a distinct relation between the uptake capacity and the growth rate could not be assessed.

Due to the preliminary nature of the reported experiments, it is not reasonable to generalize the obtained results, and in order to test the hypothesis as stated in the introduction, more research must be done with a number of different organisms grown under a variety of environmental conditions. For example Matin *et al.* (1976) reported an increase in the concentration of intermediary enzyme systems with decreasing growth rates. A *Pseudomonas* spec. and a *Spirillum* spec. were grown in a chemostat culture under a lactate limitation. A decrease of the growth rate was accompanied by an increase of the concentration of lactate dehydrogenase, aconitase and glucose-6P-dehydrogenase.

SUMMARY

Bacterial strain HIS 42 was grown in a histidine-limited chemostat at growth rates of 0.01, 0.05 and 0.1 $h^{-1}$. On samples taken from this culture the uptake of a number of organic compounds was measured with $^{14}C$-labelled substrates. Bacterial strain HIS 53 was grown in an aspartate-limited chemostat at growth rates of 0.003 and 0.01 $h^{-1}$. On samples from this culture the uptake capacity for several amino acids was determined by measurement of the oxygen consumption rate at saturating substrate concentrations.

At the applied culture conditions the utilization of a number of organic compounds could be demonstrated, indicating that a simultaneous uptake of several organic compounds under natural conditions is very likely. Furthermore, the experiments demonstrated that the uptake capacity for a certain substrate increases with the increase of the specific growth rate, as far as the growth limiting substrate is concerned. This increase represents the adaptive behaviour of the organisms to the higher growth rate. For the other substrates a distinct relation between the uptake capacity and the growth rate could not be assessed.

Table 1. The uptake of several organic compounds by HIS 42 in relation to the specific growth rate.

| | | | | |
|---|---|---|---|---|
| Specific growth rate | 0.01 | 0.05 | 0.10 | $h^{-1}$ |
| Uptake rate of histidine in the culture | 10.0 | 50.0 | 100.0 | $\mu mol\ l^{-1}\ h^{-1}$ |
| Measured maximum uptake rate of histidine | 18.6 | 49.6 | 57.8 | " |
| Maximum uptake rate of | | | | |
| aspartate | 55.7 | 73.6 | 55.7 | " |
| leucine | 1.1 | 1.0 | 0.7 | " |
| glycine | 8.4 | 10.4 | 6.4 | " |
| glucose | 1.5 | 1.3 | 1.1 | " |
| acetate | 5.0 | 3.7 | 9.5 | " |
| amino acid mixture | 134.4 | 195.8 | 188.5 | $\mu gat\ C\ l^{-1}\ h^{-1}$ |

Table 2. The uptake of several organic compounds by HIS 53 in relation to the specific growth rate.

| | | | |
|---|---|---|---|
| Specific growth rate | 0.003 | 0.01 | $h^{-1}$ |
| Uptake rate of aspartate in the culture | 4.0 | 12.0 | $\mu mol\ l^{-1}\ h^{-1}$ |
| Measured maximum uptake rate of aspartate | 18.7 | 62.0 | " |
| Maximum uptake rate† of | | | |
| alanine | 14.0 | 13.9 | " |
| serine | 23.3 | 28.3 | " |
| glutamate | 12.8 | 42.8 | " |
| asparagine | 24.6 | 62.9 | " |
| proline | 2.9 | 4.0 | " |
| histidine | 5.6 | 3.3 | " |
| arginine | 2.4 | 2.2 | " |

† The presented maximum uptake rates refer to the substrate utilized for respiration purposes only. The oxygen consumption rate was converted to an uptake rate for the substrate in question assuming a full respiration of the substrate.

Key-words: heterotrophic bacteria, activity, organic compounds, simultaneous uptake.

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