Marine Biology 41, 19-28 (1977)

MARINE BIOLOGY

Distribution of Colorless Sulfur Bacteria (*Beggiatoa* spp.) in a Coastal Marine Sediment

B.B. Jørgensen

Institute of Ecology and Genetics, University of Aarhus; Aarhus, Denmark

Abstract

The populations of *Beggiatoa* species were quantified in the sediments of a brackish fjord (Limfjorden, Denmark) over a period of 1 year. The bacterial distribution was compared to the physical structure of the sediment, the redox profile, the concentration and production rate of H_2S, and the rate of O_2 consumption. The bacteria are absent in fine and in medium-grained sand, but in muds which are aggregated in faecal pellets very high population densities are found throughout the year with average biomasses of 5 to 20 g m^{-2}. The bacteria seldom form coatings on the surface, but are distributed within the upper few centimeters of oxic sediment. They receive H_2S partly from the lower anoxic layers and partly from reduced microniches scattered in the oxidized zone. Estimates of their metabolic rates indicate that *Beggiatoa* spp. may play a significant role in the sulfur and carbon cycles of the investigated sediments.

Introduction

Colorless sulfur bacteria of the genus *Beggiatoa* are among the largest and most conspicuous of all bacteria. Their multicellular filaments range in width from 1 to 50 µm and may reach a total length of 1 cm. They occur commonly in both freshwater and marine environments where they are mainly found on the surface of sediments or on decomposing plant material. On these solid surfaces they are motile by means of a gliding mechanism similar to that of *Oscillatoria* spp. and other blue-green algae.

When growing in the presence of H_2S and O_2, *Beggiatoa* species store granules of elemental sulfur inside their cells. In nature, the filaments only seem to grow where both these compounds are present. Since H_2S is not stable in oxic waters due to auto-catalytic oxidation by O_2, the habitat of *Beggiatoa* is restricted to the transition zone between oxic and anoxic environments where O_2 and H_2S are continuously supplied by diffusion along opposite gradients. Where these gradients are steep, *Beggiatoa* and other types of colorless sulfur bacteria may form white patches of dense cell masses. Such habitats are often designated "sulfureta" due to the visible dominance of sulfur bacteria. They are, however, restricted to rather few, shallow areas rich in decomposing organic material.

The physiology of the genus *Beggiatoa* is poorly known, partly due to difficulties in cultivating these organisms (Faust and Wolfe, 1961; Scotten and Stokes, 1962). They were originally considered to be true chemoautotrophs, deriving energy from the oxidation of H_2S to sulfuric acid via intracellular sulfur and deriving organic carbon from the fixation of CO_2 (Winogradsky, 1888). Since the pioneer work of Winogradsky, the autotrophic nature of *Beggiatoa* has repeatedly been questioned. Pure cultures have been grown on organic media with, for example, acetate as the carbon source both with and without H_2S (Pringsheim, 1964, 1967; Kowallik and Pringsheim, 1966). Although the oxidation of H_2S seems to stimulate growth even in a rich organic medium, it still remains to be demonstrated how much energy *Beggiatoa* gains from this process (Kuenen, 1975).

Little is known about the ecological importance of *Beggiatoa* spp. and related organisms in aquatic ecosystems. The thiobacilli are generally assumed to be the organisms mainly responsible for the biological oxidation of inorganic sulfur

in nature (Ivanov, 1968; Sokolova and Karavaiko, 1968). They can be isolated from most sediments, waters, and soils and often occur in high numbers. However, an extensive investigation of the distribution of the other colorless sulfur bacteria has been lacking. Due to their recognizable morphology, these types are ideal for direct quantification in contrast to most other bacteria. This fact was utilized in the present study. *Beggiatoa* filaments were counted and measured under the light microscope and their biomass was calculated. The bacterial distribution was compared to the physical-chemical structure of the sediment.

Materials and Methods

Sampling and Counting of Beggiatoa

The populations of *Beggiatoa* species were studied in a shallow, brackish fjord, Limfjorden, situated in the northern part of Denmark. The area has no significant tide. The salinity fluctuates between 23‰ and 29‰, while the water temperature varies from 0° to 20°C throughout the year. Sediment samples were obtained from the central parts of the fjord at water depths of 4 to 12 m. The sampling stations are plotted on the map in Fig. 1. Station numbers 1-9 are in accordance with those of Jørgensen (1977a) where a more detailed description of the physical-chemical properties and the sulfur metabolism of the sediments is given. The selected stations comprise a gradient of sediment types ranging from fine sand in the northern, exposed area (Stations 1 and 2) to soft mud in the southern, sheltered parts.

Sediment material was collected with a "Haps" corer (Kanneworff and Nicolaisen, 1973). With Plexiglass tubes of 26 mm internal diameter these samples were divided into a number of subcores each retaining an undisturbed, vertical stratification of the original sediment. The subcores were brought back to the laboratory and kept at the *in situ* temperature until analysis was initiated the next day.

For bacterial counting, 20 to 40 mg subsamples of fresh sediment were consecutively removed from the central part of a core at 5 to 10 mm intervals while the sediment was gradually extruded from the tube. Each subsample was placed on a microscope slide in a few drops of seawater and weighed. It was then carefully smeared over the whole slide. This preparation was then studied under a normal light microscope. After initial practice, all motile *Beggiatoa* filaments containing intracellular sulfur could be identified at 62 x magnification. Their length was measured using a calibrated ocular micrometer and their width was similarly measured at 400 x magnification. The biomass of each filament was then calculated assuming a cylindrical shape and a density of 1 g cm^{-3}. From the vertical distribution of biomass the population density could be calculated per unit area of sediment.

A special procedure was used when the bacteria grew in dense plates on the surface of the sediment. In this case it was not possible to distinguish and measure individual filaments in the tangled web; the bacteria had to be dispersed first. A small, defined area of the mat was removed by careful preparation and placed on a glass slide covered by a thin film of seawater. The slide was then placed in a Petri dish on moist filter paper. A few grains of Na_2S were placed on the paper and the Petri dish was covered by a lid. In this simple chamber, *Beggiatoa* was supplied with both O_2 and H_2S and usually the filaments would start to glide out of the entangled mat and spread evenly over the slide. After 5 to 10 h, all filaments could then be counted and measured under the microscope without difficulty.

Although these counting techniques are rather time-consuming, they are probably not more so than other direct or viable counting techniques. They have the great advantage of giving absolute numbers of bacteria since each individual is readily identified due to the presence of intracellular sulfur droplets. Although *Beggiatoa* may grow in cultures without storing sulfur, such hyaline forms were not found in the investigated sediments in any significant numbers.

The relative standard deviation of single sample counts was ± 50% when parallel cores were compared. The variance was largely due to heterogeneous distribution of the bacteria.

The *Beggiatoa* populations were quantified five times during one year on 9 permanent stations in Limfjorden. A few other localities were sampled on single occasions. A total number of about 10,000 filaments were measured.

Other Determinations

The redox profile of the sediment was measured in undisturbed cores with a platinum electrode as described by Fenchel (1969).

Hydrogen sulfide in the porewater was measured by iodine titration (Golterman,

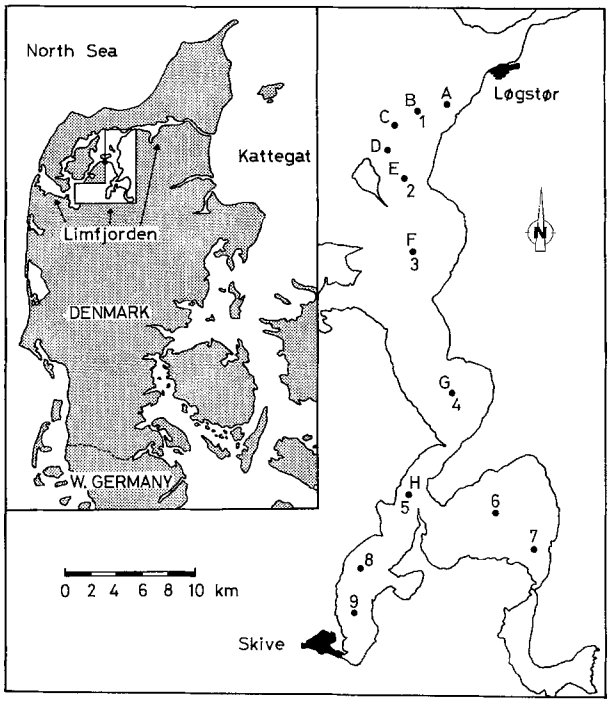

Fig. 1. Map of investigation area, Limfjorden, Denmark. Permanent sampling stations are numbered 1-9; other stations (A-H) were sampled only once

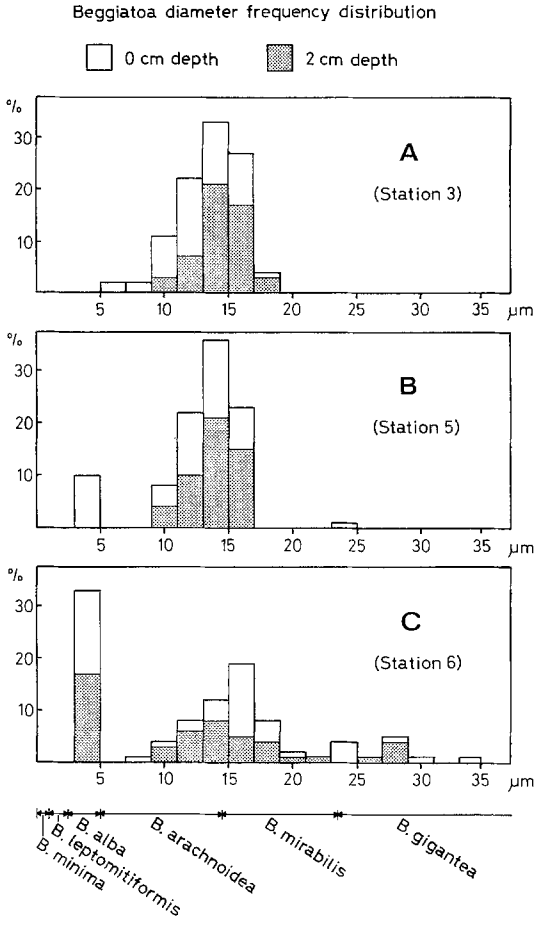

Fig. 2. *Beggiatoa* spp. Frequency distribution of diameters of bacteria from 3 different sediment samples. 50 filaments from surface and from 2 cm depth, respectively, were measured and frequency is given as percent in 2 µm size-intervals. Filament widths of the 6 recognized species are indicated at bottom of figure

1971). Porewater was obtained by pressure filtration through a membrane filter (Millipore, 0.45 µm pore size). The water was squeezed out of 20 g of sediment under 3 atm of N_2 pressure and directly collected in 5 ml of 2% $CdCl_2$.

Elemental sulfur (S^o) was determined in dense patches of *Beggiatoa* and other colorless sulfur bacteria. A known area of the bacterial plate was sampled and extracted in 96% ethanol. The amount of elemental sulfur was then determined spectrophotometrically from the absorbance at 260 nm (Gemerden, 1967).

The rate of bacterial H_2S production in the sediment was measured with a radio-tracer technique described by Jørgensen and Fenchel (1974). The rate of O_2 consumption by the benthic community was measured in undisturbed cores as described by Pamatmat (1971). The data were obtained from Jørgensen (1977a).

Results and Discussion

Size Distribution and Systematics of Beggiatoa

From the beginning of the present study it was attempted to classify the populations of *Beggiatoa* to species level. The systematics of the genus is almost exclusively based upon the width of the filaments. From this character, 6 species have been recognized. In addition, a number of *species incertae sedis* are described (Buchanan and Gibbons, 1974). In pure

culture studies, Winogradsky (1888) and Pringsheim (1964) have shown that the filaments of the various isolated *Beggiatoa* strains have constant widths during growth even under varying physiological conditions.

The natural populations were found to constitute a continuum of size ranges. Fig. 2 shows the diameter frequency distribution of 100 filaments at three different sampling localities. The size intervals of the 6 recognized species are also indicated. The three examples illustrate typical size-distribution patterns of the area. In Population A, the size range was rather narrow with a distinct maximum at 15 µm. All other populations investigated had a similar frequency maximum around 15 µm. Since the two species *Beggiatoa arachnoidea* and *B. mirabilis* are separated at exactly this width they cannot be distinguished in these natural populations.

Population B again has a size group centered around 15 µm, but in addition a distinct group of 3 to 5 µm is recognized. In Population C, the 3 to 5 µm group is even more pronounced while the larger filaments cover a wide size range from 7 to 35 µm. Filaments larger than 26 µm are recognized as *B. gigantea* but these do not separate markedly from *Beggiatoa mirabilis*. Only the 3 to 5 µm population of *B. alba* stands out as an isolated group. In order to make an ecologically useful classification of the larger forms, new characters in addition to the cell diameter must be applied. The length of the individual cell segments was found to give no additional group separation, but it was observed that the largest filaments (>25 µm width) often had an irregular contour. This, however, was not consistent and may be due to poor physiological condition.

The three examples in Fig. 2 were measured in January. In summer the populations at the same localities showed a very similar size distribution. The three sediment stations differed only little in physical structure, chemistry, and biological activity, and no explanation can therefore be given for the constant size differences.

In Fig. 2 both populations from the very sediment surface and from 2 cm depth were studied. There was a general tendency for the smaller forms to be relatively more abundant at the surface. Thus, in the *Beggiatoa arachnoidea/B. mirabilis* group there was an increase of 2 to 3 µm in the mean diameter from the surface to 2 cm depth. The small *B. alba* often occurred only at the surface.

All the measurements of filament dimensions are summarized in Table 1,

Table 1. *Beggiatoa* spp. Size characteristics of bacteria from Limfjorden. Average dimensions of 3 size classes are shown together with minimum and maximum sizes for whole population

	Diameter (µm)	Length (mm)	Biomass (g)
3-5 µm group, mean	4	0.8	$1.6 \cdot 10^{-8}$
5-23 µm group, mean	14	1.0	$1.5 \cdot 10^{-7}$
>23 µm group, mean	26	1.9	$1.0 \cdot 10^{-6}$
Minimum	3	0.1	$0.7 \cdot 10^{-9}$
Maximum	35	5.0	$2 \cdot 10^{-6}$
Variation	11-fold	50-fold	3,000-fold

which gives the average and extreme sizes of *Beggiatoa* species in Limfjorden. The >23 µm filaments are distinguished for practical reasons, but do not comprise a well separated group. It is noticeable that no individuals smaller than 3 µm width were found, although they were often searched for under higher magnification. Thus, *B. minima* and *B. leptomitiformis* do not occur in these sediments although they should be common in marine environments (Lackey, 1961).

All filaments were longer than 100 µm and a few reached up to 5 mm in length. There was a 3000-fold biomass difference between the smallest ($0.7 \cdot 10^{-9}$ g) and the largest ($2 \cdot 10^{-6}$ g) forms. In comparison, the average bacterial biomass in aquatic ecosystems is about 10^{-12} g per cell (Sorokin and Kadota, 1972). Thus, *Beggiatoa* spp. falls within the size range of the interstitial microfauna and the large microalgae of the sediments rather than within the range of other bacteria (cf. Fenchel, 1969). However, in contrast to these, *Beggiatoa* spp. are multicellular organisms with filaments containing a few hundred cells. The biomass of the individual cells is $2 \cdot 10^{-11}$ to $1 \cdot 10^{-8}$ g.

Vertical Distribution

Beggiatoa spp. occurred abundantly in the uppermost few centimeters of the sediments. Vertical distribution was found to be closely correlated with chemical stratification. Since all filaments were observed to contain intracellular sulfur granules they indicated that both H_2S and O_2 were available.

Hydrogen sulfide is produced in the anoxic sediment mainly by bacterial reduction of sulfate. In the zone where O_2 and H_2S meet, rapid oxidation will take place. In sterile, oxygenated sea-

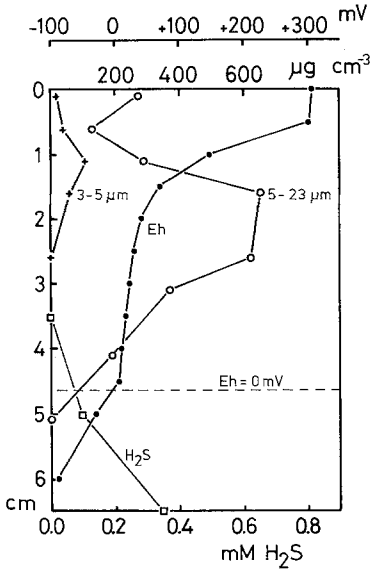

Fig. 3. *Beggiatoa* spp. Vertical distribution of 3 to 5 µm and 5 to 23 µm size groups (µg cm^{-3}) in sediment (Station 3, March 1974). Redox profile and depth of Eh = 0 mV are also shown, together with H_2S concentration of the porewater

water, the half-life of H_2S is in the order of 0.5 h (Almgren and Hagström, 1974). Therefore, the horizon where both H_2S and O_2 are present in sediments is usually very narrow if their mixing is governed by vertical diffusion alone.

The steepest gradients are obtained at the surface of completely reduced sediments, where H_2S reaches the oxygen of the open waters. This is the condition which leads to mass development of *Beggiatoa* species and other sulfur bacteria at the sediment-water interface. By developing dense bacterial plates on top of the sediment they even steepen the chemical gradients by impeding microturbulent mixing (Jørgensen and Fenchel, 1974). Since the colorless sulfur bacteria are always competing with the chemical sulfide oxidation, this growth form is likely to increase the quantity of sulfide which they can exploit.

Studies on *Beggiatoa* populations in nature have almost exclusively been restricted to cases where they grow in visible density. However, in the Limfjorden sediments, this growth form only occurred in short periods of the summer when the stagnant bottom waters became partly or totally depleted of oxygen. This caused the reducing zone to rise to the very sediment surface, although H_2S seldom appeared in the bottom water. The change was observed during SCUBA diving as a blackening of the mud surface followed by the appearance of a fine white coating a few days later. In such periods *Beggiatoa* plates covered up to tens of km^2. But in general the occurrence of *Beggiatoa* spp. in the sediment was quite inconspicuous, although it was one of the most dominating organisms present. Fig. 3 shows as an example the vertical distribution of two size classes during winter. The 3 to 5 µm group of *B. alba* is found closer to the sediment surface under more oxidizing conditions than the larger forms. This was found always to be the case when both size-classes were present in the same sediment.

The narrow and the wide forms have their maximum population densities at depths of 1 and 2 cm, respectively, below the surface. There is also a small maximum at the very surface, but nothing like the dense plates just mentioned. The distribution pattern in Fig. 3 was very typical for sediments with a deep oxic zone of 3 to 6 cm thickness. The redox curve in Fig. 3 indicates that the porewater is highly oxidized only in the uppermost 1 cm. From 1 to almost 5 cm depth the redox potential is poised at 0 to +100 mV. In this intermediate zone, where both H_2S and O_2 must be available for *Beggiatoa*, the maximum biomass is reached. Oxygen penetrates down into this zone by diffusion and bioturbulence in the porewater. Hydrogen sulfide is produced in the reducing sediment below 5 cm. Only in this deeper layer is there any detectable concentration of H_2S.

There is, however, also a high production rate of H_2S from sulfate in the upper 5 cm (Jørgensen, 1977b). The sulfate reducers, which are strict anaerobes, are here growing within anoxic micro-environments in the oxidized sediment. Such reduced microniches were found to be present as approximately 100 µm large clumps of sediment. These are whole or partly disintegrated faecal pellets of benthic invertebrates. Hydrogen sulfide which is produced within the pellets rapidly diffuses out into the surrounding porewater which contains oxygen. It does not accumulate to detectable concentrations, but is oxidized chemically or by *Beggiatoa* and other sulfide-oxidizing bacteria which grow in the interstitial pores.

The filaments never penetrated the microniches where they would lack oxygen. Neither were they found within the completely reduced sediment where H_2S was present in increasing concentrations but where O_2 was lacking.

In the intermediate zone, where *Beggiatoa* reached the highest densities, the redox potential was usually poised within a narrow Eh range of 0 to +100 mV.

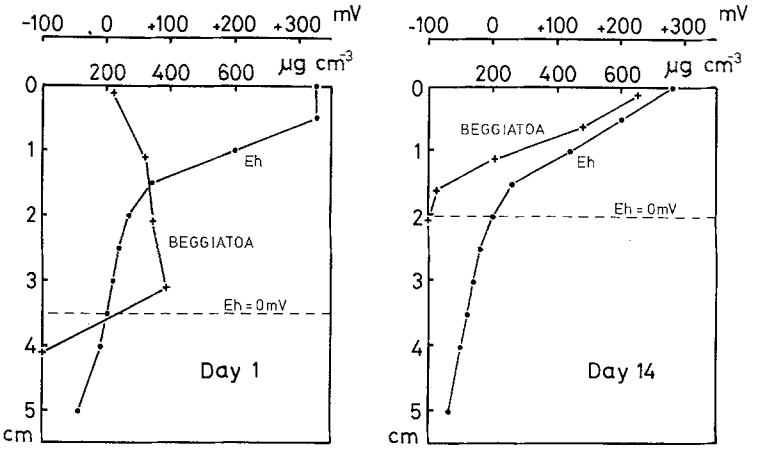

Fig. 4. Vertical distribution of *Beggiatoa* spp. biomass and redox potential in sediment 1 and 14 days after sampling

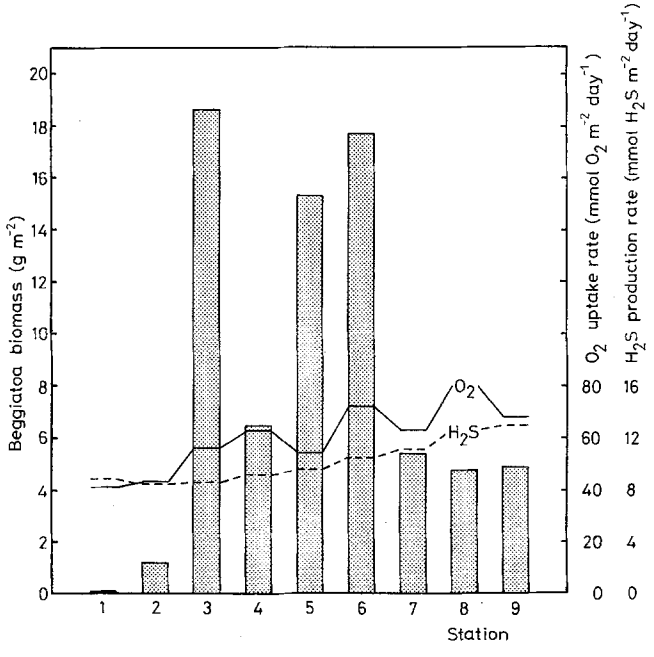

Fig. 5. *Beggiatoa* spp. Yearly average biomass on 9 sampling stations. Rates of O_2 uptake and H_2S production in upper 10 cm of sediment are also shown

Table 2. *Beggiatoa* spp. Average and maximum population densities of 3 size classes in uppermost 1 cm of sediment from Limfjorden

Diameter (µm)	Individuals cm^{-3}		Biomass (µg cm^{-3})	
	Mean	Maximum	Mean	Maximum
3-5	1,000	8,000	20	150
5-23	3,000	20,000	500	3,000
>23	<10	500	<10	500

This range seems to be buffered by a low oxygen content of the pore water and an extremely low H_2S concentration. The microniches themselves are not registered by the redox measurements due to their small size.

The vertical distribution of *Beggiatoa* spp. was compared to the redox profile in 40 sediment cores from Stations 3 to 9. The lower boundary for the bacteria generally coincided with the 0 mV level in the sediment, -10 mV being the mean value. Only in sediment layers below the depth of 0 mV was H_2S present in the porewater in detectable concentrations.

The thickness of the oxidized surface layer varied throughout the year from a few millimeters in summer to 3-5 cm in winter. The lower boundary to which *Beggiatoa* species occurred followed the depth of the 0 mV isovolt through the seasons. In periods when there was a rapid change in the redox gradient, *Beggiatoa* migrated up or down in the sediment. Thus in summer periods, when the bottom water rapidly became depleted of oxygen, they appeared on the sediment surface within a few days in spite of their low growth rate. The generation time in pure culture is in the order of 1 day (Winogradsky, 1888).

Vertical migration was demonstrated in the laboratory, where intact sediment cores were left in the dark. Due to decreased water turbulence the reducing zone expanded upwards and caused the bacteria to follow. Fig. 4 shows the distribution of *Beggiatoa* spp. and the Eh just after sampling the sediment and 2 weeks later. The depth of 0 mV ascends

from 3.5 to 2 cm and so does the lower boundary of *Beggiatoa*. Assuming the distributional change to be due only to migration, the average moving distance of the filaments is 1 to 2 cm. Crozier and Stier (1926) found the gliding speed of *Beggiatoa* on agar plates to be 0.4 to 1.2 cm h^{-1} in the temperature range 5° to 20°C. Thus, the bacteria have the ability to rapidly change their position in response to changing gradients.

Table 2 summarizes results on the bacterial densities; the average and maximum population sizes in the uppermost 1 cm of the sediment (Stations 1 and 2 are not included) are shown. The numbers of filaments are quite low compared to other bacteria, but due to their large size the biomass is considerable. Total bacterial numbers in coastal sediments are generally in the range of 10^8 to 10^9 cells per cm^3 (Dale, 1974). Direct counts under the epifluorescence microscope after Acredine orange staining gave numbers in the Limfjorden sediments of 1 to $4 \cdot 10^8$ bacteria cm^{-3} (Jørgensen and Perry, in preparation). With an average size of 1 μm^3 the bacterial biomass, excluding *Beggiatoa*, is only 100 to 400 µg cm^{-3}, or less than the average biomass of *Beggiatoa*.

Population Sizes, Seasons, and Sediment Types

From the vertical distribution of *Beggiatoa* spp., the total biomass can be calculated per unit area. This was done for all stations and seasons, and the following results are expressed as g wet weight per m^2.

In Fig. 5 the yearly average biomass of *Beggiatoa* spp. is shown for each sampling station. The averages range from 0.03 to 19 g m^{-2}, with the lowest values occurring in the northernmost, sandy sediments (Stations 1 and 2). In the soft muds (Stations 3-9), the biomass is around 5 to 20 g m^{-2}, and even up to 48 g m^{-2} have been measured on single occasions. The southern stations (3-9) represent an area covering 150 km^2. The total biomass of the benthic fauna in this area is in the order of 50 g m^{-2} (J. Birklund, personal communication). Thus, *Beggiatoa* constitutes a considerable part of the total benthos on a weight basis and, due to its small size, probably accounts for an even larger part of the community metabolism.

As previously discussed, the supply of both O_2 and H_2S are important for the growth of *Beggiatoa* in nature. Fig. 5 compares the biomass distribution with the yearly average of oxygen uptake of the sediment and of H_2S production from bacterial sulfate reduction. Both aerobic and anaerobic respiration are seen to increase from north to south by approximately 60%. Thus, the size of the *Beggiatoa* population shows little correlation with the supply of O_2 and H_2S and is probably more strongly regulated by other sediment parameters.

The largest biomass changes were found in the transition area between sandy and soft sediments (Stations 1-3). In this area also the species composition changed from a population of only 3 to 5 µm wide filaments (*Beggiatoa alba*) in the sand to a more mixed population dominated by larger forms in the mud. A closer study was therefore made of the bacterial distribution in relation to the physical structure of the sediment.

Eight sediment stations (A-H) were selected, comprising a gradual change of sediment type (Fig. 6). In the northernmost, exposed area the sediments are composed of well-sorted, medium to fine-grained sand (Stations A and B). Further south the area becomes increasingly sheltered from waves and currents and silt is mixed into the sand. The sand fraction gradually changes from 100% to an insignificant proportion between Stations B and F. There is a concomitant decrease in the median grain size of the sand from 190 to 70 µm and less.

Apart from the grain size distribution there is an important shift in the sediment properties between Stations E and F. All the northern stations have a homogeneous sediment structure. But the muds at Station F and further south are all aggregated in faecal pellets. As previously discussed, some of these pellets form reduced microniches in the oxidized sediments and thus produce excellent growth conditions for *Beggiatoa* species.

The population size and composition react sharply to this change in sediment structure. In the well sorted, fine sand of Stations A-C the biomass is less than 0.01 g m^{-2} and the few individuals which are found are all of 3 to 5 µm width. In the sediments of mixed composition (D-E) the biomass increases to around 0.1 g m^{-2} and a few larger forms are present among the filaments. But going to the pellet mud of Station F and further south, the biomass suddenly increases 100-fold and the large filaments become totally dominating. Since the frequency of 3 to 5 µm forms in Fig. 6 is based on numbers, the relative importance of these small filaments in the muds is even smaller on a biomass basis.

Our present knowledge of the physiology and ecology of *Beggiatoa* spp. is not sufficient to explain the observed distribution pattern. It seems to be neither a direct function of the total supply of

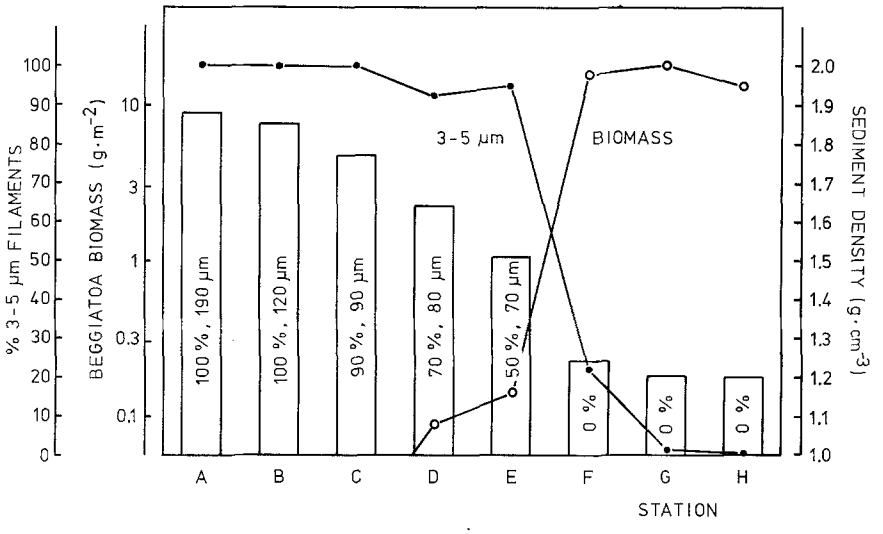

Fig. 6. Sediment properties and *Beggiatoa* spp. distribution on Stations A-H (March, 1974). Bars indicate density of sediment. Inside bars is given weight % and median grain size of the sand fraction. The two curves show total biomass of *Beggiatoa* and the relative frequency of 3 to 5 µm filaments

H_2S and O_2 (cf. Fig. 5) nor of the thickness of the oxidized sediment zone, at least not on a macro-scale. The thickness of the oxidized surface layer is slightly deeper in the northern than in the southern sediments, but the difference is small compared to the seasonal variation.

The physical and the microchemical structure seem to be the main factors regulating the interstitial growth of *Beggiatoa*. The microniche structure probably explains the high biomass present in the muds. The absence of large forms in the sandy sediments may be due to the small dimensions of the interstitial spaces in a manner similar to that found for the interstitial microfauna by Fenchel (1969). Thus, the 10 to 20 µm wide filaments may easily move through the loose pellet structure of the muds but not through the fine sand with rigid interstices which are more or less filled with silt. In these habitats a few small forms may still find space.

This pore-space hypothesis is supported by the observation that the large forms are often abundant down to several centimeters depth in coarse-grained sands. sands.

The seasonal variation in the *Beggiatoa* population size shows no regular trend (Fig. 7). The maximum biomass is reached in July and the minimum in October. There is little difference in the redox structure and the metabolic rate of the sediment between these two months. Both the supplies of O_2 and of H_2S to the upper sediment layer have a very regular, seasonal variation. No explanation can be given for the 3-fold decrease of biomass between May 1974 and May 1975, but the difference is statistically significant (the relative standard deviation of the menas is ± 15%).

The 3 to 5 µm forms contributed a small but rather constant fraction of the total population throughout the year. The largest forms (>23 µm width) appeared mainly in winter, when they showed up on most of the southern stations.

Other Colorless Sulfur Bacteria

All types of colorless sulfur bacteria which store intracellular sulfur granules should be detectable by the same procedure as applied to *Beggiatoa* spp. During the present study, such forms were only found during summer periods when the bottom waters became stagnant and almost depleted of oxygen. This caused a mass kill of the benthic fauna. On the surface of the decomposing animals grew a mixture of colorless sulfur bacteria which, in addition to *Beggiatoa* spp., were mainly comprised of *Thiovulum* sp. *Thiovulum* formed fine veils, especial-

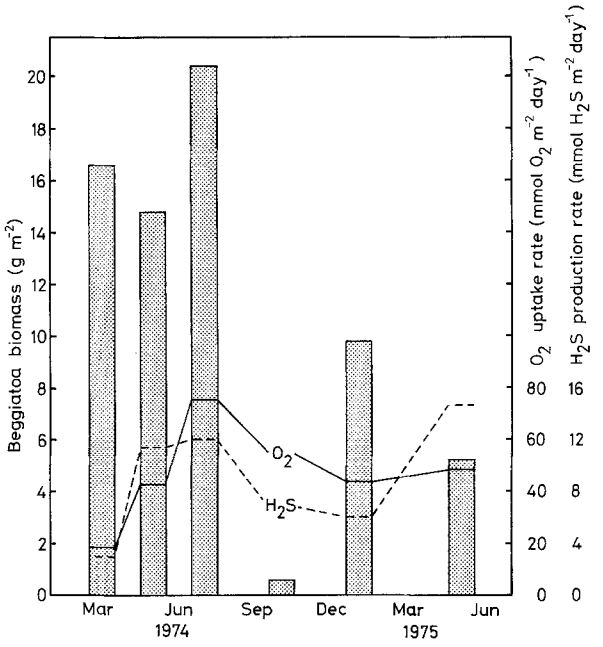

Fig. 7. *Beggiatoa* spp. Seasonal variation of biomass and of rate of O_2 uptake and H_2S production in upper 10 cm of sediment. Results are averages of Stations 1-9

ly over the large banks of dead mussels (*Mytilus edulis*). By SCUBA diving it was estimated that *Thiovulum* veils could occasionally cover areas of up to several km^2. Often the floating *Thiovulum* veils were overgrown with *Beggiatoa* which thus gained mechanical support to move to the zone of optimal H_2S-O_2 gradients.

By careful manipulation it was possible to bring *Thiovulum* veils under the microscope and to count the bacteria. In their most pure growth form, the density of *Thiovulum* sp. was 10^5 to 10^6 cells cm^{-2} with $5 \cdot 10^5$ cells cm^{-2} as a mean value. Since the ovoid cells are very large, 9 µm in diameter, the corresponding biomass amounts to 1.6 g m^{-2}, which is almost comparable to that of *Beggiatoa*. The bacterial density corresponds to half the maximum packing capacity for a single cell layer. Their projected area is about 40% of the total area of the veil.

The *Thiovulum* sp. cells were generally rich in sulfur granules. The total content of elemental sulfur in one veil was 0.65 µmol S cm^{-2}, corresponding to 10% of the biomass of the bacteria. However, a considerable fraction of the sulfur occurred as extracellular granules.

Conclusions

Since almost no work has been done previously to quantify natural populations of *Beggiatoa* species, it is not possible to say whether the very high densities found in Limfjorden are unique to coastal marine sediments. The only similar, quantitative study known to the author was made by Reimers (1976) in the Bay of Kiel and the Schwentine Estuary on the Baltic coast of Germany. Her findings were very similar to those in the present study with regard to the population size of *Beggiatoa*. This indicates that *Beggiatoa* may be of general, quantitative importance in estuarine sediments. In contrast to most other bacteria it can be counted by a simple and accurate procedure and it is therefore well suited for further population studies.

Unfortunately, the physiology of the bacteria is still too incompletely known to evaluate the importance of *Beggiatoa* in the sulfur and carbon budgets of the sediments. If, however, the main part of the free energy from oxidation of H_2S is utilized for bacterial chemosynthesis this may contribute significantly to the organic production of the ecosystem. Thus, Jørgensen (1977a) found that the H_2S production in the Limfjorden sediments was equivalent to 50% of the total respiratory metabolism of the benthic community. If an equivalent of half of this sulfide is converted into bacterial biomass by autotrophic growth, this will correspond to one fourth of the organic input to the sediment.

A rough estimate of the potential metabolic rate of *Beggiatoa* spp. may be obtained from their active biomass. Since only motile filaments containing sulfur granules were counted, the active biomass is equal to the total, registered biomass. An average metabolic rate for an organism of the size of *Beggiatoa* is 10 cal g^{-1} h^{-1} (Hemmingsen, 1960) or approximately 1.5 mmol O_2 g^{-1} day^{-1}. With a biomass of 10 g m^{-2}, the *Beggiatoa* population will have an estimated respiration of 15 mmol O_2 m^{-2} day^{-1}. This is one fourth of the total community respiration (cf. Fig. 5).

The possible role of *Beggiatoa* spp. for the sulfur cycle may also be estimated in the following way. The intracellular sulfur granules account for a few percent of the cell volume as estimated from microscopic observations. With a total biomass of 10 g m^{-2} the amount of intracellular sulfur is thus 0.2 to 0.5 g m^{-2}. Direct chemical measurements of S^o in *Beggiatoa* patches from the surface of Limfjorden sediments gave results in the same range. In laboratory cultures of *Beggiatoa* growing with H_2S and O_2, Winogradsky (1888) found that when the bacteria were deprived of H_2S they would oxidize all the intracellular sul-

fur within 24 h. If the turnover time for the intracellular sulfur in the natural populations is also assumed to be 1 day, the oxidation rate will be 0.2 to 0.5 g S m^{-2} day^{-1} or 5-15 mmol S m^{-2} day^{-1}. The daily rate of H_2S production is 10 mmol S m^{-2} day^{-1} (cf. Fig. 5).

Although these calculations do not show the actual rates of bacterial metabolism, they do indicate that the *Beggiatoa* populations have the potential to oxidize all the H_2S produced and thus contribute significantly to both the sulfur and the carbon cycles of the sediments.

Acknowledgements. The present study was supported in part by grants from Statens Naturvidenskabelige Forskningsråd. The tedious procedure of counting *Beggiatoa* spp. was largely carried out by E. Glob and A. Therkelsen. They are both thanked for their patient efforts.

Literature Cited

Almgren, T. and I. Hagström: The oxidation rate of sulfide in sea water. Wat. Res. *8,* 395-400 (1974)

Buchanan, R.E. and N.E. Gibbons (Eds.): Bergey's manual of determinative bacteriology, 8th ed. 1246 pp. Baltimore: The Williams & Wilkins Company 1974

Crozier, W.J. and T.J.B. Stier: Temperature characteristics for speed of movement of thiobacteria. J. gen. Physiol. *10,* 185-193 (1926)

Dale, N.G.: Bacteria in intertidal sediments: factors related to their distribution. Limnol. Oceanogr. *19,* 509-518 (1974)

Faust, L. and R.S. Wolfe: Enrichment and cultivation of *Beggiatoa alba.* J. Bact. *81,* 99-106 (1961)

Fenchel, T.: The ecology of marine microbenthos. IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities, with special reference to the ciliated Protozoa. Ophelia *6,* 1-182 (1969)

Gemerden, H. van: On the bacterial sulphur cycle of inland waters, 110 pp. Thesis, University of Leiden 1967

Golterman, H.L. (Ed.): Methods for chemical analysis of fresh waters, 188 pp. IBP Handbook No. 8. Oxford: Blackwell Scientific Publications 1971

Hemmingsen, A.M.: Energy metabolism as related to body size and respiratory surfaces, and its evolution. Rep. Steno meml Hosp. *9,* 1-110 (1960)

Ivanov, M.V.: Microbiological processes in the formation of sulfur deposits, 298 pp. Jerusalem: Israel Program for Scientific Translations 1968

Jørgensen, B.B.: The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnol. Oceanogr. (In press). (1977a)

- Bacterial sulfate reduction within reduced microniches of oxidized marine sediments. Mar. Biol. *41,* 7-17 (1977b)
- and T. Fenchel: The sulfur cycle of a marine sediment model system. Mar. Biol. *24,* 189-201 (1974)

Kanneworff, E. and W. Nicolaisen: The "Haps", a frame supported bottom corer. Ophelia *10,* 119-128 (1973)

Kowallik, U. and E.G. Pringsheim: The oxidation of hydrogen sulfide by *Beggiatoa.* Am. J. Bot. *53,* 801-806 (1966)

Kuenen, J.G.: Colourless sulfur bacteria and their role in the sulfur cycle. Pl. Soil *43,* 49-76 (1975)

Lackey, J.B.: Occurrence of *Beggiatoa* species relative to pollution. Wat. Sewage Wks *108,* 29-31 (1961)

Pamatmat, M.M.: Oxygen consumption by the seabed. IV. Shipboard and laboratory experiments. Limnol. Oceanogr. *16,* 536-550 (1971)

Pringsheim, E.G.: Heterotrophism and species concept in *Beggiatoa.* Am. J. Bot. *51,* 898-913 (1964)

- Die Mixotrophie von *Beggiatoa.* Arch. Mikrobiol. *59,* 247-254 (1967)

Reimers, T.: Anoxische Lebensräume. Struktur und Entwicklung der Mikrobiozönose an der Grenzfläche Meer/Meeresboden, 134 pp. Thesis, Zoological Institute, University of Kiel 1976

Scotten, H.L. and J.L. Stokes: Isolation and properties of *Beggiatoa.* Arch. Mikrobiol. *42,* 353-368 (1962)

Sokolova, G.A. and G.I. Karavaiko: Physiology and geochemical activity of thiobacilli, 283 pp. Jerusalem: Israel Program for Scientific Translations 1968

Sorokin, Y.I. and H. Kadota (Eds.): Microbial production and decomposition in fresh waters, 712 pp. IBP Handbook No 23. Oxford: Blackwell Scientific Publications 1972

Winogradsky, S.: Beiträge zur Morphologie und Physiologie der Bacterien. I. Zur Morphologie und Physiologie der Schwefelbacterien, Leipzig: Arthur Felix 1888

Bo Barker Jørgensen
Institute of Ecology and Genetics
University of Aarhus
Ny Munkegade
DK-8000 Aarhus C
Denmark

Date of final manuscript acceptance: December 3, 1976. Communicated by T.M. Fenchel, Aarhus