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A regulated river ecosystem in a polluted section of the Upper Vistula*

4. Biomass and bacterial decomposition

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A b s t r a c t — The author determined the biomass and respiration of heterotrophic bacteria, indicating the temporary accumulation of energy at the level of a bacterial population or the amount of energy released from the environment as a result of destruction processes. Production was highest in summer for most groups of bacteria, and in autumn for proteolytic ones. On the basis of biological and chemical indices and a 4 grade classification it was confirmed that the water of the studied section of the Vistula is heavily polluted.

K e y w o r d s: regulated river, pollution, bacteria, biomass, respiration.

1. Introduction

Throughout the biosphere, organic matter is constantly being biologically transformed (R h e i n h e i m e r 1980, S e k i 1982). A key component of its cycling in aquatic ecosystems are microorganisms (B a b i c h, S t o t z k y 1983), of which bacteria play the most significant role (K a n g, S e k i 1983). Not only does bacterial biomass make it possible to asses the loading of the environment with living organic matter, which constitutes a source of food for the organisms of other trophic links (B a b i c h, S t o t z k y 1983), but it also indicates the amount of energy temporarily eliminated from cycling. Respiration, in turn, is a measure of the energy removed from the environment and is evidence of the destruction and mineralization of organic matter (O d u m 1982), i.e. of processes of the self-purification of water.

The aim of the work was to determine the biomass and respiration of heterotrophic bacteria and to assess the quality of the water of a 25 km section of the Vistula on the basis of bacterial and chemical indices.

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2. Study area, material, and method

The study was carried out between November 1982 and December 1983, at six stations (fig. 1), whose location and detailed description has been given by Dumnicka, Kownacki (1988a).

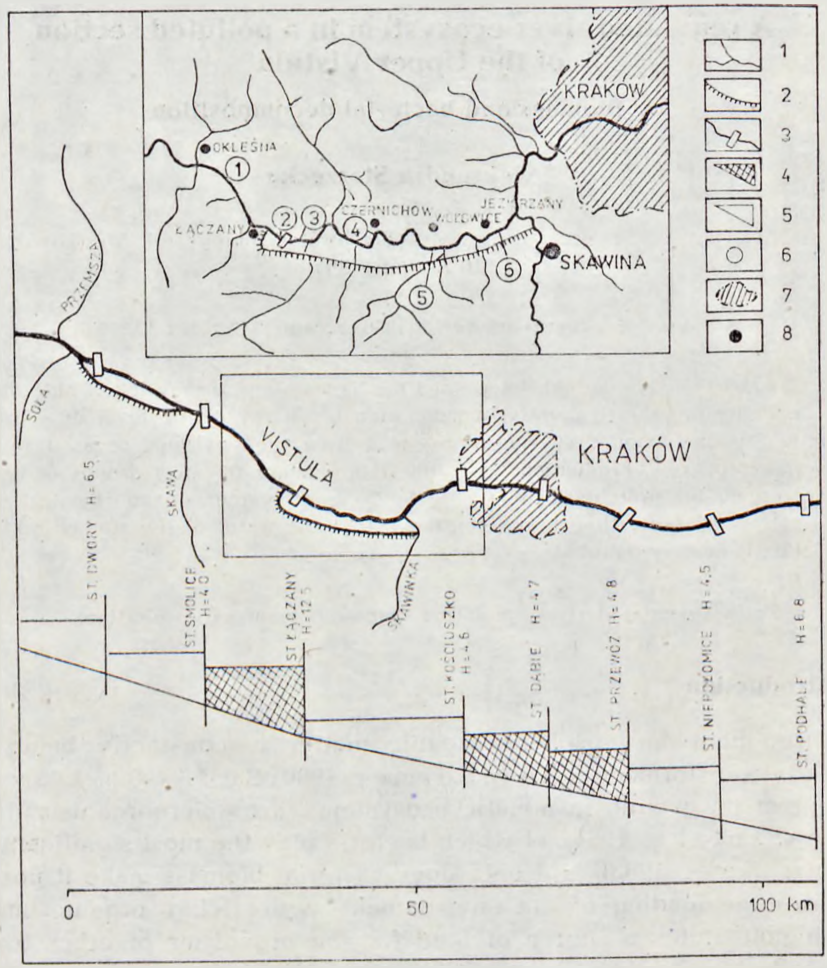

Fig. 1. The Upper Vistula, showing cascade building and stations on the investigated river section. 1 — rivers; 2 — canal; 3 — water stages, dams; 4 — water stages built; 5 — water stages under construction and planned; 6 — stations; 7 — cities; 8 — towns and villages

Samples for the study were taken on 11 dates from 25—30 cm below the water surface, using a bathometer of the author's own construction (Starzecka 1975). Altogether 66 samples were collected, and the following determinations were made: the total number of heterotrophic

bacteria and the number of proteolytic bacteria — using the plate method — and the titre of the bacteria taking part in nitrogen and sulphur cycles (ammonifying and denitrifying bacteria, those releasing H_2S during the decomposition of proteins). Titre values were recalculated into numbers according to Mc Crady's statistical tables (Collins, Lyne 1970; Rodina 1968). The substrates and media used were given by Starzecka (1979).

In order to calculate the bacterial biomass (B), expressed in mg dry weight per dm^3 of water, the mean annual number of heterotrophic bacteria per cm^3 of water (L), obtained by the plate method, and coefficient (a) were used:

$$B = L \cdot a \text{ (mg dry wt } dm^{-3} \text{ of water)}$$

where:

$a = c \cdot 10^3 \cdot 10$

where:

$c = 1.6 \cdot 10^{-10}$ — the dry weight of 1 cell in mg, according to Kuznyetsov and Romanyenko (1963);

10^3 — the calculation of the mean number of heterotrophic bacteria from 1 cm^3 of water to 1 dm^3 of water;

10 — the tenfold underestimation of the plate method taken into account.

Finally the bacterial biomass (B) was calculated according to the formula:

$$B = 1.6 \cdot 10^{-6} \cdot L \text{ (mg dry wt } dm^{-3} \text{ of water)}$$

Assuming 1 mg dry weight equals 5 cal, the bacterial biomass expressed in calories (B') equals:

$$B' = 5 \cdot B \text{ (cal } dm^{-3} \text{ of water)}$$

The respiration of the bioseston (R_{bs}) expressed in mg O_2 dm^{-3} of water $diel^{-1}$ was calculated from the BOD_5 values, using calculation coefficients q and d:

$$R_{bs} = BOD_5 \frac{1}{q \cdot d} (O_2 \text{ mg } dm^{-3} \text{ diel}^{-1})$$

where:

q — the temperature correction factor calculated according to Krogh's growth curve (Vinbyerg 1956; Shushchyenya 1972) is a variable for converting the data obtained at a temperature of 20°C to the temperature at the time of sampling,

d — a constant coefficient that permits calculation from BOD_5 of the quantity of oxygen used by the bioseston during the first 24 h (Streeter, Phelps 1925).

Hydrolytic biochemical decomposition takes place according to a monomolecular reaction, i.e. the quantity of organic compounds oxygenated in each identical unit of time is proportional to that remaining unoxygenated. It was determined that, in a unit of time, BOD_5 changes

by a certain constant value, which at 20°C amounts to 20.6% of the total BOD_5. In accordance with this, each successive day, owing to microbial activity, the amount of oxygen in the environment decreases by 20.6% with respect to the initial oxygen concentration on that day. It can be calculated that during five successive days the initial oxygen concentration falls by 68.4%. These two values made it possible to calculate coefficient d:

$$d = \frac{68.4}{20.6} = 3.3$$

The final formula takes the form:

$$R_{bs} = \frac{BOD_5}{3.3 \cdot q} (O_2 \text{ mg dm}^{-3} \text{ diel}^{-1})$$

Assuming that 1 mg O_2 equals 3.38 cal, the respiration of the total bioseston in calories (R'_{bs}) equals:

$$R'_{bs} = 1.02 \frac{BOD_5}{q} (\text{cal dm}^{-3} \text{ diel}^{-1})$$

In order to calculate the respiration of the seston bacteria (R_b) the number of heterotrophic bacteria (L) was used, from the data for the River Vistula, determined for 1 cm^3 of water and recalculated per 1 dm^3 and from the data on the respiration of mixed populations of heterotrophic bacteria for the River Nida (author's own unpublished results)

$$R_b = L \cdot 10^3 \cdot r_k \text{ (mg } O_2 \text{ dm}^{-3} \text{ diel}^{-1})$$

where:

$$r_k = \frac{r_N}{L_N \cdot 10^4} (\text{mg } O_2 \text{ cell}^{-1} \text{ diel}^{-1})$$

where:

r_k — the respiration of 1 cell

r_N — the respiration of a particular population of heterotrophic bacteria from the River Nida (mg O_2 dm^{-3} $diel^{-1}$)

$L_N \cdot 10^4$ — the number of bacteria calculated per dm^3 of water, taking into account the correction factor for the tenfold underestimation of the data obtained using the plate method.

The mean value of $r_k = 4.2 \cdot 10^{-9}$ mg O_2 $cell^{-1}$ $diel^{-1}$ was calculated from 188 measurements of the respiration of heterotrophic bacteria, obtained in a two year study carried out on a eutrophicated section of the River Nida (unpublished data).

$$R_b = 4.2 \cdot 10^{-9} \cdot L \text{ (mg } O_2 \text{ dm}^{-3} \text{ diel}^{-1})$$

Assuming that 1 mg O_2 = 3.38 cal, the respiration of mixed populations of heterotrophic bacteria in calories R'_b amounts to:

$$R'_b = 3.38 R_b$$

In order to compare the magnitude of the respiration of the bacteria

Jørgensen's coefficient (1979) was also used, according to which the oxygen consumption of the bacteria of meadow soils at a temperature of 20°C is $7.0 \cdot 10^{-9}$ mm³ O_2 cell^{-1} h^{-1}. Oxygen volumes were expressed in terms of weight, using the conversion factor of 0.0014 mg O_2 equivalent to 1 mm³ O_2 at a temperature of 11°C (the annual mean for the Vistula). Further recalculating Jørgensen's coefficient according to the titre for r_k, the value $2.4 \cdot 10^{-9}$ mg O_2 cell^{-1} diel^{-1} was obtained, i.e., a coefficient close to the value obtained in the calculations used in the case of the Nida (unpublished data).

The quality of the water was determined on a 4 grade scale (purity classes I to IV) and 10 indices (5 biological and 5 chemical) (Starzecka et al. 1979; Starzecka 1984). The final quality of the water was determined by the numerical taxonomic value of pollution (NTVP), which is the mean of the sum obtained from the numerical values from 1.0 to 4.0 assigned to the indices, for purity classes I to IV, respectively. The values of NTVP calculated with an accuracy of 0.01 facilitate comparison of the quality of the water within the same class of purity.

3. Results

3.1. Number and biomass of heterotrophic bacteria

The greatest number and biomass of bacteria (annual means) were found above the reservoir at Łączany (Station 1), and within it (Station 2). At the stations lying below (Stations 3—5) the values were about halved or smaller, being lowest at Station 5. At Station 6 an increase in the number and biomass of the bacteria was noted (Table I).

Table I. Number and biomass of heterotrophic bacteria at Stations 1–6. Sp – Spring; Su – Summer; Au – Autumn; Wi – Winter

Station	Annual means			Seasonal means											
	total number of bacteria	dry weight		total number of bacteria				dry weight							
								mg dm^{-3}				cal dm^{-3}			
	10^5 cm^{-3}	mg dm^{-3}	cal dm^{-3}	Sp	Su	Au	Wi	Sp	Su	Au	Wi	Sp	Su	Au	Wi
1	5.2	0.832	4.16	3.1	12.0	3.4	2.2	0.496	1.920	0.544	0.352	2.48	9.60	2.72	1.76
2	4.0	0.640	3.20	1.3	30.0	2.3	2.6	0.208	4.800	0.368	0.416	1.04	24.00	1.84	2.08
3	2.2	0.352	1.76	2.5	2.1	1.5	2.7	0.400	0.336	0.240	0.432	2.00	1.68	1.20	2.16
4	2.3	0.368	1.84	3.4	2.1	1.0	3.2	0.544	0.336	0.160	0.512	2.72	1.68	0.80	2.56
5	2.0	0.320	1.60	2.6	1.9	1.1	2.5	0.416	0.304	0.176	0.400	2.08	1.52	0.88	2.00
6	2.9	0.464	2.32	3.8	4.6	0.9	3.2	0.480	0.736	0.146	0.512	2.40	3.68	0.72	2.56

Analysis of the data concerning particular seasons shows that the development of the heterotrophic bacteria was greatest in summer also at the two upper stations (1.2), a 2.5-fold higher bacterial biomass being

found at Station 2. With the course of the river the changes in number and biomass were similar to those in the average annual means. No such regularity was observed at other times of the year. In spring the smallest biomass of heterotrophic bacteria was found in the water of the reservoir (Station 2), while in autumn and winter this occurred at Stations 6 and 1, respectively (Table I).

3.2. Seasonal changes in the biomass of bacteria differing in biochemical capacity

The biomass production of all the groups of bacteria identified was highest in summer, except for the proteolytic ones whose biomass reached its highest values in autumn (fig. 2A). The biomass of the proteolytes increased constantly downriver, reaching a maximum at Station 6 (fig. 2A). Throughout the year the biomass production of the ammonifying bacteria and of those releasing H_2S during the decomposition of proteins were the most stable at Station 1. Greater fluctuations on this river section were noted in the group of denitrifying bacteria, this being

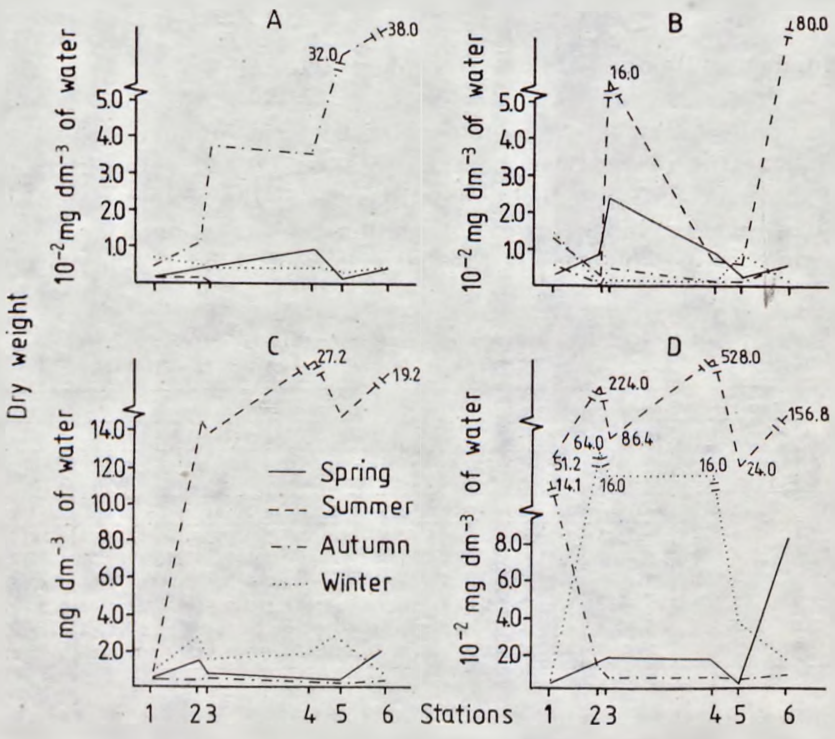

Fig. 2. Seasonal changes in bacterial biomass. A — proteolytic bacteria; B — H_2S releasing bacteria; C — ammonifying bacteria; D — denitrifying bacteria

reflected in their more than twofold increase in biomass production in summer and autumn, as compared with other times of year (fig. 2B, C, D). The greatest biomass of bacteria releasing H_2S during the decomposition of proteins was found at Station 3 in spring and summer and at Station 6 in summer (fig. 2B), while for ammonifying and denitrifying bacteria this was observed at Station 4 in summer (fig. 2C, D).

3.3. The respiration of seston organism, including heterotrophic bacteria

Respiration of the bacteria and of the phyto- and zooplankton was greatest in the upper part of the studied section of the Vistula (Stations 1, 2, 3). Further downriver (Stations 4, 5) the values for the whole bioseston were respectively 1.3—1.5 times smaller than at Stations 1 and 2

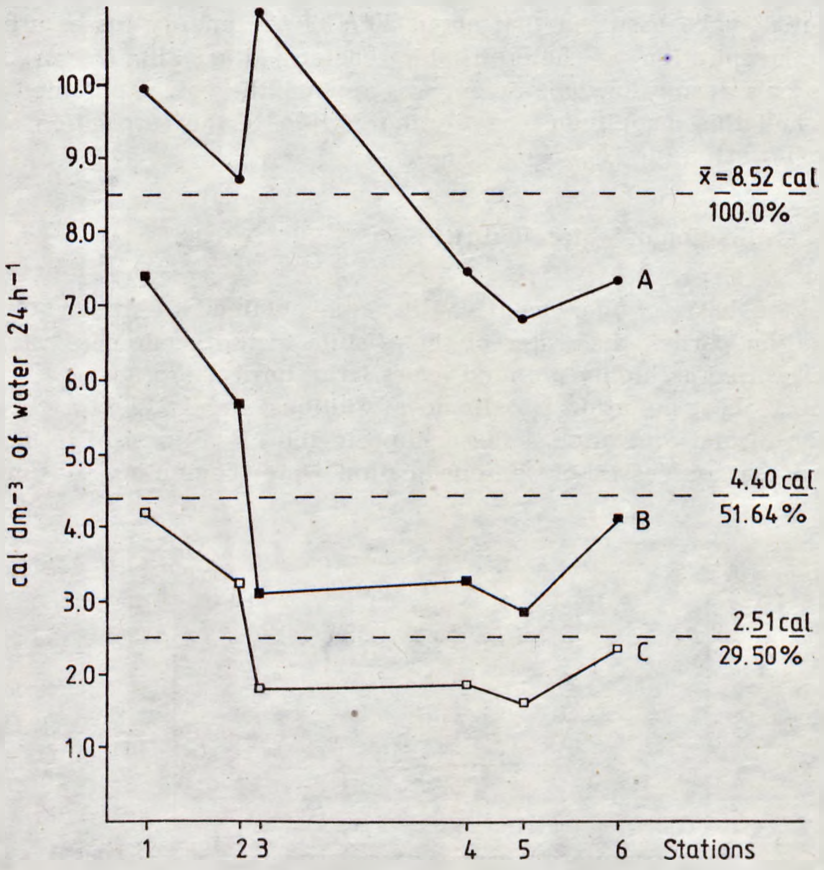

Fig. 3. Respiration of A — seston organisms, B, C. — heterotrophic bacteria and mean values with percentage share

above and within the reservoir and Station 3 below the dam in Łączany. In the last section (Station 6) there was an increase in the respiration of the bioseston (fig. 3A).

In the case of the bacteria, the highest values of respiration were found at Stations 1 and 2. In contrast, at Station 3 there was a distinct, more than twofold, fall in their respiration, as compared with Station 1, and an almost twofold one with respect to Station 2. Further down the Vistula the bacterial respiration remained at a similar level and was smallest at Station 5, while at Station 6 there was an increase in both the respiration of the bacteria and in that of the other components of the bioseston (fig. 3B).

As a result of the respiration of the whole bioseston, the mean energy losses in the investigated section of the Vistula were 8.52 cal dm^{-3} of water $diel^{-1}$. Assuming this value to be 100% and taking the respiratory coefficient calculated on the basis of the author's own (unpublished) results, it may be assumed that about 50% of the energy losses are due to the respiration of heterotrophic bacteria (fig. 3B). When Jørgensen's respiratory coefficient was applied, the values obtained were about half this magnitude, constituting 29.50% of the respiration of the whole bioseston (fig. 3C).

3.4. Classification of water quality

On the basis of the mean NTVP value obtained for the whole investigation period, the water of the Vistula in the section investigated was classified as highly polluted (class III of purity) (fig. 4).

The highest pollution was found at Stations 1 and 2. Below the dam (Station 3) and at Station 4 the decline in the NTVP of 0.20 to 0.23 indicated that processes of self-purification were beginning to function

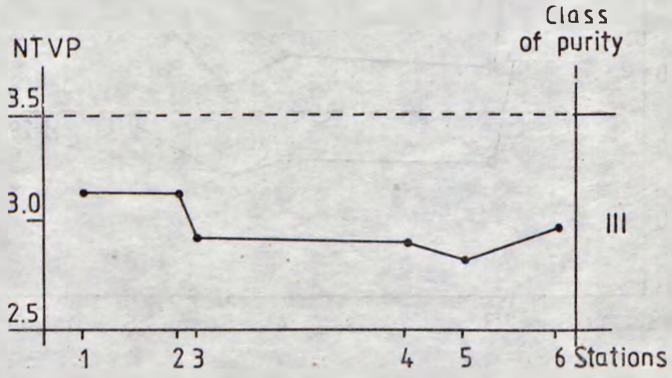

Fig. 4. Classification of water purity. NTVP — numerical taxonomic values of pollution

downriver, being most pronounced at Station 5 (a decline in pollution of 0.30). At Station 6 the increase in the biological and chemical indices of water quality evidenced higher pollution of the water.

4. Discussion

Bacteria play the main role in the assimilation of dissolved organic matter in natural waters. This is connected with their wide capacity to adapt to various conditions of the environment, combined with the enormous metabolic diversity of particular groups of bacteria, which make possible the transformation and degradation of various substances occurring in that environment.

Several of the studies concerning the changes in heterotrophic activity are based on the uptake of radio-labelled carbon (^{14}C) included as part of the experimental substrate (Stanier et al. 1966, Parsons, Strickland 1962, Hobbie, Crowford 1969, Williams, Leb 1973, Wright, Hobbie 1966), or on measurement of the level of adenosine triphosphate (ATP) in cells, indicating an increase or decrease in bacterial activity (Droste, Sanchez 1983). All the determinations carried out under laboratory conditions carry a considerable error, since they make no allowance for the great abundance and diversity of dead organic matter present in the natural environment, or for the simultaneous cooperation of many biotic and abiotic factors that affect the rate of reactions. Even determinations as precise as ATP depend to a considerable degree on the physiological condition and properties of a microorganism (Harrison, Maitra 1968, cit. Odum 1982). Determining the number of bacteria by the plate method and then inferring on such a basis the size of the biomass may lead to incorrect interpretation of the metabolic activity of mixed populations of heterotrophic bacteria (Droste, Sanchez 1983). This method does, however, give the possibility of obtaining comparative results useful in the determination of relative numbers and of the size of the biomass in a given volume of water. It also reflects the state of loading of a given habitat with living organic matter and indicates the temporary accumulation of energy at the population level. Quantitative measurements of the oxygen consumed by a given population in cultures of sampled natural (not enriched) water, however, indicate metabolic activity and permit conclusions to be drawn as to the destruction and elimination of energy from the environment.

The calculation coefficients used in the present study, based on the data of Kuznyetsov and Romanyenko (1963), Jørgensen (1979), and the author's unpublished studies, have made it possible to assess the energy flow at the level of the bacterial microflora in the strongly polluted environment of the River Vistula (NTVP correspond-

ing to purity class III). The greatest loading of the studied section of the River Vistula with organic matter occurred at the two upper Stations (1, 2). Here, the organic matter underwent the highest degree of bacterial destruction (65—74%), and also the energy accumulated at the level of the population of heterotrophic bacteria was greatest. The heavy pollution of the river at these two stations is evidenced — besides the bacteriological indices — by the low content of oxygen dissolved in the water, by a high content of organic matter, a high BOD_5, and a high content of ammonia nitrogen, chorides, and total phosphorus (Kasza 1988).

Below the dam (Station 3) there was a distinct decline in the total biomass of the group of heterotrophic bacteria and, in contrast to the whole bioseston, a marked decrease in the number of heterotrophic seston bacteria. With a doubled oxygenation of the water and only a slight fall in the content of organic matter, as compared with the upper stations, this decrease is difficult to explain. It is true, however, that there was a rise in the biomass of bacteria with various biochemical capacity, and this might indicate the presence of forms of organic and inorganic matter conducive to increasing the rate of various enzymatic processes. Nevertheless, in the total balance these values did not bring about an increase in the biomass of the heterotrophic bacteria. The lower release of energy from the environment at the bacterial level, observed at Station 3, is probably compensated for by the action of other seston or benthic organisms. The greatest energy losses, as shown by Dumnicka and Kownacki (1988b), took place as a result of the respiration of oligochaetes, which found conditions favourable for their growth at Station 3, despite the considerable changes in their number and biomass during the year. The distinct increase in the trophicity of the water at Station 3 (Bednarz 1988) indicated smaller processes of bacterial decomposition. For all this, the decrease in the bacterial indices of pollution and in some chemical ones, with a much higher content of oxygen dissolved in the water resulting from the impoundment, pointed to a marked effect of self-purification processes (a decrease in NTVP), which were most pronounced at Station 5, where the river's original character was best preserved. At Station 6 there was an increase in the indices of pollution (a rise in NTVP), evidencing the inflow of fresh quantities of pollution and nutrients. The phenomenon was also confirmed in the algological and zoological investigations (Bednarz 1988, Bednarz, Żurek 1988; Kwandrans 1988; Dumnicka, Kownacki 1988b).

In the strongly polluted section of the Vistula (purity class III) bacterial processes of destruction and the resulting release of energy were, on average, 52% compared with the energy released owing to the life processes of the remaining components of the bioseston (phyto- and zooplankton). It seems that this value, though only estimated, is quite

realistic. This is supported by the investigations of Zo Bell (1946), which show that heterotrophic bacteria mineralize over half the organic substances present in an aquatic environment. The development of heterotrophic bacteria depends on many factors, the most important being nutrients, temperature, and oxygen (Overbeck 1974; Jones 1977). According to one opinion, in a strongly eutrophicated environment, rich in nutrient substances, temperature is of secondary importance only (Griffiths et al. 1978; Krashyennikova 1960; Drabkova 1965). Such a view is also supported by the high percentage (65—74%) of bacterial destruction obtained in the present study at the upper stations (1 and 2), which showed the greatest amounts of organic substances and nutrients and a water temperature similar to that of the stations lying lower down. Moreover, the seasonal fluctuations in the production of bacterial biomass do not always coincide with the periods with the highest temperature. This might indicate a considerable influence of specific substances which are substrates for the development of groups of bacteria differing in biochemistry. Other phenomena, such as those resulting from reciprocally antagonistic relationships between the seston organisms, cannot be ruled out.

Comparison of the energy losses calculated on the basis of the two coefficients used here (r_k, personal data and Jørgensen's) in spite of distinct differences also shows the results to be realistic. Higer values of destruction (1.7 on average) obtained for the data on aquatic bacteria are more plausible, since they were calculated for a eutrophicated equatic environment. The values obtained according to Jørgensen's coefficient (1979) might be underestimated because the respiratory coefficient was calculated for a soil environment where, as a rule, there are more bacteria per unit weight or volume, hence the destruction itself, calculated per cell, may be lower. The percentage variations in bacterial destruction in the studied section of the Vistula, from 28.5 to 74.25, seem likely. On the basis of Efford's data (1969) (cit. Odum 1982) the small organisms (bacteria, algae, protozoans) obtained from in situ studies of the bottom biocenosis of a small lake in Western Canada increase the respiration coefficient of the whole biocenosis the most, where about 30% constitutes bacterial respiration. Moreover, from numerous investigations (Donderski 1983; Strzelczyk, Mielczarek 1971) it can be seen that aquatic bacteria are more active than the benthic ones and utilize nutrient compounds to a greater degree, this being connected with the greater availability of readily assimilated nutrient substances in the water than in the sediment (Henrici 1939; Waksman 1941; Skopintsyev 1949).

5. Polish summary

Ekosystem uregulowanego i zanieczyszczonego odcinka Górnej Wisły

4. Biomasa i destrukcja bakteryjna

Badania nad wodami Wisły przeprowadzono w okresie od listopada 1982 r. do grudnia 1983 r., na 6 stanowiskach usytuowanych pomiędzy 33 i 58 km biegu rzeki (ryc. 1).

Celem pracy było określenie biomasy i oddychania bakterii heterotroficznych oraz ocena jakości wody na 25 km odcinku Wisły na podstawie wskaźników biologicznych i chemicznych.

Ogólna liczba bakterii heterotroficznych i ich biomasa były największe w lecie na dwóch górnych stanowiskach (1, 2) powyżej zbiornika i w samym zbiorniku (tabela 1). Z biegiem rzeki (od st. 3 do 5) obserwowano spadek liczebności i biomasy heterotrofów, w przeciwieństwie do st. 6, gdzie stwierdzono wzrost tych wielkości (tabela 1).

Produkcja biomasy oznaczanych grup bakterii była największa w lecie, z wyjątkiem bakterii proteolitycznych, których biomasa osiągnęła największe wartości w jesieni (ryc. 2A, B, C, D). Najbardziej wyrównaną produkcję biomasy bakterii rozkładających substancje białkowe obserwowano na stanowisku 1 (ryc. 2B, C). Większe wahania na tym stanowisku stwierdzono w grupie bakterii denitryfikacyjnych, na co wskazywał 2-krotny wzrost produkcji ich biomasy w lecie i jesieni — w odniesieniu do pozostałych okresów roku (ryc. 2D).

Destrukcja w wyniku oddychania całego biosestonu, w tym również bakterii heterotroficznych, była największa na stanowiskach 1 i 2. Natomiast poniżej progu wodnego (st. 3) przy utrzymujących się wysokich wartościach respiracji fito- i zooplanktonu nastąpił wyraźny spadek oddychania i tym samym destrukcji bakteryjnej (ryc. 3).

Straty energii w wyniku respiracji biosestonu wynosiły 8,52 cal dm^{-3} wody $doba^{-1}$, przy czym ponad 50% uwalniania energii zachodziło w wyniku metabolicznej aktywności bakterii heterotroficznych (ryc. 3).

W wyniku przeprowadzonej na podstawie wskaźników biologicznych i chemicznych klasyfikacji, potwierdzono silne zanieczyszczenie Wisły na badanym odcinku. Liczbowe wartości taksonomiczne zanieczyszczenia — LWTZ, w obrębie wszystkich 6 stanowisk odpowiadały III klasie czystości i wahały się w granicach 2,84 do 3,14 (ryc. 4).

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