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A LABORATORY ASSESSMENT OF THE PURIFICATION OF AGRICULTURAL WASTE WATER CONTAINING A HIGH ORGANIC LOAD USING ALGAE

1. INTRODUCTION

Abundant data is available on the use of microorganisms in the purification of agricultural waste water. Due to the increasing number of industrial cattle- and pig-breeding farms, the problem of efficient waste water disposal is becoming more urgent. The feaces of domestic animals are rich in organic compounds which should be decomposed before the waste water is discharged into rivers. Such decomposition requires fermentation tanks where the bacterial and protozoic degradation of these organic compounds is possible. The addition of algae accelerates the biological oxidation process, and at the same time removes organic compounds [9]. Protein can be obtained from algae cultivated on decomposing organic substances (not the case in the traditional oxidation ditch). This system is called a "High-Rate Oxidation Pond" — HIROP. Various kinds of waste water are employed as media, the algae used belonging either to the Chlorophyta or the Cyanophyta. Mixed algae-bacteria systems are also used [8].

The author's own work has shown that algae such as Chlorella and Spirulina grow well on hen manure extracts, the medium being substantially purified at the same time. It was desided to carry out similar experiments with pig manure and the mixotrophic alga Chlorella pyrenoidosa, strain No. 366, Scenedesmus acutus, strain No. 449, and two halophytic forms: the cyanophyte Spirulina platensis and the Chlorophyta Chlorella sp. var. halofila. The object of the study was to establish conditions for cultivating these algae so as to obtain an intensive biomass production and, at the same time, a high degree of purification of the medium used.

2. MATERIAL AND METHODS

Algae. The following algae (from the Zator Zootechnical Institute's Collection of Algae) were used in the experiments: Chlorella pyrenoidosa Chick., strain No. 366, Scenedesmus acutus Meyne, strain No. 449, Spirulina platensis (Gom.) Geitl. and Chlorella sp. var. halofila [2].

Medium. Aqueous pig manure extracts (PME) were used for the cultures. Solid faeces were collected from bedding-free pig sheds, dried to constant weight and thoroughly homogenised. Extracts were then prepared by shaking dry sample of manure with appropiate amounts of cold distilled water for 24 h. After centrifuging, the supernatant liquid was filtered through filter paper. The extracts were used as the medium for algae culture. The following concentration gradients of PME (in g/cm^3) were used: 1 : 20, 1 : 30, 1 : 40, 1 : 50 (i.e. one part by weight of manure: given volume of water). The mineral nutrient medium L_{5m} [6] and the so-called "Zator" medium [7] were used as controls.

Culture. Algae were cultivated in 0.5 l flasks containing 250 cm^3 of the culture medium. The intensity of illumination was 5.5 klx, and the cultures were aerated continuously with a mixture of air and 5% CO_2. In some experiments, a light intensity gradient from 2.5 to 80 klx was applied. Cultures were also grown for periods ranging from 14 days down to 36 h, depending on the conditions.

Estimations. The algal growth was measured by the increase in dry mater. Proteins and mineral contents of the biomass obtained were determined. In addition, the PME were analysed for: pH-value, turbidity (determined by measuring optical density at maximum extinction with λ=500 nm); organic nitrogen content (determined by Kjeldahl's method); total phosphorus (molybdenum method), sulphur (weight method), magnesium (titration method) and iron (thiocyanate method), the procedures being those normally applied in waste water analyses [5]. Organic matter in the PME was determined from its permanganate value (PV) and from an estimation of the biochemical oxygen demand (BOD) [5]. All these analyses were performed before and after culture, and after centrifuging the algae. The analytical differences indicated the extent of purification of the medium.

3. RESULTS AND DISCUSSION

3.1. Effect of PME dilution on the biomass increase of Chlorella and bacterial flora

Preliminary experiments showed that Chlorella pyrenoidosa No. 366 grows well in aqueous PME concentrations of from 2 to 5%. Higher concentrations do not give better results as the solutions obtained are

Table 1. Contents of biogenic elements in L_{5m} medium and individual PME solutions [mg/dm³]

Tab. 1. Zawartość biogennych pierwiastków w pożywce L_{5m} i w poszczególnych roztworach EKS [mg/dm³]

No. Nr	Dilution rate manure : water Rozcieńczenie kał : woda	mg/dm³				
		N	P	Mg	S	Fe
1	1 : 20	428.5	386.0	190.0	23.6	1.10
2	1 : 30	278.6	214.0	118.0	20.7	1.01
3	1 : 40	185.2	171.0	97.0	12.8	0.87
4	1 : 50	169.2	147.0	80.0	11.3	0.72
5	L_{5m}	151.5	45.5	14.8	19.5	0.60

PME – aqueous pig manure extracts
EKS – ekstrakt kału świńskiego

very turbid. A dilution gradient of 1 : 20, 1 : 30, 1 : 40 and 1 : 50 was used to determine the optimum PME concentration for growth of this alga. Table 1 presents the content of biogenic elements in these PME and in the mineral (L_{5m} nutrient) controls.

The concentration of biogenic elements in all extracts was many times greater than in the mineral control. Only the sulphur content was somewhat lower in the two greatest dilutions. Algal growth was therefore expected to be good in all the media, also because of the low turbidity of the PME. Chlorella pyrenoidosa No. 366 was cultivated in these

Table 2. The increases of Chlorella pyrenoidosa No. 366 and bacteria biomasses gained in 14-day cultures on various PME solutions and L_{5m} medium; light intensity – 5.5 klx, constant aeration with 5% CO_2 addition, inoculum: 120 mg d.w./dm³

Tab. 2. Przyrost biomasy Chlorella pyrenoidosa nr 366 i bakterii na poszczególnych EKS i L_{5m} w trakcie 14-dniowej hodowli w oświetleniu 5.5 klx, przy nieprzerwanym przewietrzeniu z dodatkiem 5% CO_2, inoculum: 120 mg s.m./dm³

No. Nr	Dilution rate manure : :water Rozcieńczenie kał : woda	Manure concentration Stężenie kału [%]	Total biomass Biomasa całkowita [g/dm³]	Bacteria + precipitate Bakterie + osad	Algae biomass Biomasa glonów	lg (number of algae cells) log. z liczby kom. glonu	pH	
							initial początkowe	final końcowe
1	1 : 20	5.0	2.6460	0.9920	1.6540	8.32	6.8	7.6
2	1 : 30	3.3	2.3781	0.5432	1.8349	8.48	7.2	7.5
3	1 : 40	2.5	2.1873	0.7904	1.7969	8.46	7.3	7.4
4	1 : 50	2.0	2.0542	0.2860	1.7682	8.45	7.2	7.3
5	L_{5m}	–	–	–	1.3960	8.44	5.3	7.3

PME – as in Table 1
EKS – jak w Tab. 1

Table 3. Turbidity of PME solutions prior and subsequent to culture devoid of algae and with algae, after centrifugation (numbers – absorbance, $\lambda = 500$ nm)

Tab. 3. Mętność EKS wyjściowa i po hodowli bez glonów (liczby – optyczna gęstość, $\lambda = 500$ nm) i z glonami po ich odwirowaniu

No. Nr	Dilution rate Rozcieńczenie	Samples devoid of algae [days] Próbki bez glonów [doby]							Subsequent to algae culture (14th day) Po hodowli z glonami (14 dzień)	Dry weight of the precipitate Sucha masa osadu [g/dm³]
		0	2	4	7	10	12	14		
1	1 : 20	0.82	0.98	1.12	0.94	0.67	0.38	0.38	0.36	0.9920
2	1 : 30	0.62	0.70	0.82	0.73	0.41	0.30	0.30	0.24	0.5432
3	1 : 40	0.42	0.70	0.66	0.61	0.26	0.17	0.17	0.16	0.3904
4	1 : 50	0.36	0.63	0.57	0.45	0.18	0.16	0.16	0.15	0.2869

PME – as in Table 1
EKS – jak w Tab. 1

media. Separate cultures without added algae were also done to estimate the increase in biomass of bacteria, always present in large numbers in every culture growing in PME.

The algae were cultivated for 14 days in a thermostat at 29°C, illuminated at an intensity of 5.5 klx, the cultures being aerated with an air +5% CO_2 mixture. Culture growth was determined by means of dry weight and cell number. These results are given in Table 2.

The greatest increase in total biomass in the alga-bacteria system was found in the 5% medium in which the increase in bacterial biomass was also greatest. However, the greatest increase in Chlorella pyrenoidosa biomass was found in PME dilutions of from 2.5 to 3.5%. This increase could be defined precisely by counting the number of algal cells.

Turbidity changes during the bacterial cultures were measured and the results compared to the turbidity of the centrifuged PME medium after algae cultures. Table 3 presents these results, which give the optical density of the PME, measured at maximum extinction with $\lambda = 500$ nm. The samples without algae were centrifuged and the precipitated biomass given in g d.w./dm³. Between the 2nd and 4th day of culture, the turbidity of the solution increases. This is caused by rapid multiplication of bacteria which fall to the bottom at the end of this period. This phenomenon not only causes the spontaneous precipitation of organic compounds, but also leads to considerable purification of the medium, in which after 14 days' culture the turbidity was 50% less than at the outset. A considerable precipitate was obtained. For cultures in 5% PME, the

algae-free organic sediment constitutes some 37% of the total biomass obtained.

These experiments were carried out bearing in mind the possibility of obtaining a large biomass which could be used as a feedstuff, therefore, the useful properties of all the organic matter, i.e., the biomass of algae and bacteria, were considered. Depending on the PME concentration, it contains from 42 to 48% protein, which could make it a useful animal feedstuff. In the remainder of this discussion, the term "cultivated biomass" will be used to mean the biomass of algae and will not, for simplicity's sake, be broken down into algal and bacterial components. The latter is relatively constant, whereas in experiments to control the process of culture growth, the parameters refer to the optimisation of algal growth only. However, when seeking to interpret the results obtained, it ought to be remembered that some of the processes taking place are to be attributed also to bacterial metabolism, which is necessary, for instance, in the uptake of some elements from the medium.

In addition, pH changes in the media were analysed. The optimum pH for the growth of Chlorella pyrenoidosa is 7.1 - 7.4, thus both the initial and final pH values of the medium (Table 2) are convenient, since they do not deviate from the optimum values.

Taking into account such data as the greatest algal biomass increase, the relatively small increase in bacterial biomass, and the appropiate pH value, a PME dilution of 1 : 30 was thus taken as optimum and used in further experiments. It is also the concentration normally found in liquid manure [1].

Under these conditions of growth, the cultures attained a stationary phase between the 8th and 12th day, depending on the PME dilution. The growth rate reached a maximum during the first 48 h of incubation.

3.2. Degree of PME purification by Chlorella pyrenoidosa

The growth of algae in PME is accompained by an appreciable purification of the media. This was shown by the successive decrease in turbidity. The purpose of further analysis was to determine the extent to which biogenic elements are lost from the medium and to measure the decrease in content of oxidisable matter by using the permanganate method and estimating the BOD.

Table 4 shows the percentage uptake of the different elements from the PME after 14-day cultures of Chlorella pyrenoidosa in relation to the initial quantities of these elements. Thus, in comparision with initial values, 80 - 87% of nitrogen was lost, as was 27 - 44% of phosphorus, 27 - 52% of magnesium, 32 - 41% of iron and 21 - 51% of sulphur. These amounts are considerable. Indeed, elements whose concentrations in

Table 4. Uptake of elements from PME solutions in 14- day cultures of Chlorella pyrenoidosa No. 366; in per cent of loss from the initial amount

Tab. 4. Zużycie pierwiastków z EKS po 14-dniowej hodowli Chlorella pyrenoidosa nr 366, w procentach ubytku od stanu wyjściowego

No. Nr	Dilution rate manure: water Rozcieńczenie kał: woda	% loss % ubytku				
		N	P	Mg	S	Fe
1	1 : 20	80.32	27.76	52.85	21.80	39.29
2	1 : 30	80.70	44.56	49.14	27.07	32.67
3	1 : 40	83.77	31.58	37.84	30.04	41.38
4	1 : 50	87.19	31.08	40.43	51.72	44.30

PME – as in Table 1
EKS – jak w Tab. 1

PME were high showed the greatest loss. This indicates that Chlorella pyrenoidosa is capable of purifying the medium to a large extent.

The degree of PME purification was also indicated by the permanganate value which gives an idea of the extent to which the extract can be oxidised, and by the magnitude of the BOD.

In the above-described experiment, PV decreased by 65 - 69% by the end of the culture, as compared with the initial value, depending on the PME concentration. BOD fell by 92 - 95%. The decreases in these values are significant and once again demonstrate the usefulness of this alga in purifying waste water from pig farms.

3.3. Optimisation of growth in cultures of Chlorella pyrenoidosa in PME

In order to implement waste water purification using Chlorella, it is necessary to select the culture conditions such that the growth period of algae, and thus the waste water retention time, is reduced to a minimum of 1 - 2 days. Thus an attempt was made to estimate the optimum growth conditions of Chlorella pyrenoidosa in PME.

The effect of light intensity was determined using the optimum PME dilution of 1 : 30. The following light intensity gradient was used: 2.5, 5, 10, 20, 30, 50 and 80 klx. The growth intensity of the algae cultures was measured by the increase in dry matter, the degree of purification of the medium being estimated by the decrease in its turbidity and PV; the results are presented in Table 5.

It was found that the growth rate of the cultures increases with light intensity, reaching a maximum at intensities of from 50 to 80 klx. The

Table 5. Effect of light intensity on the growth of Chlorella pyrenoidosa No. 366 cultivated on PME solution in dilution 1 : 30 (measured as dry weight increase) and decrease of the medium contamination (measured by turbidity and PV value). Culture duration — 14 days, inoculum 140 mg d.w./dm³.

Tab. 5. Wpływ natężenia światła na wzrost glonu Chlorella pyrenoidosa nr 366 na EKS w rozcieńczeniu 1 : 30, mierzony wartością suchej masy, oraz towarzyszący temu wzrostowi spadek zanieczyszczenia podłoża mierzony jego mętnością i wartością TN. Czas hodowli 14 dni, inoculum 140 mg s.m./dm³.

No. Nr	Light intensity Natężenie światła [klx]	Biomass Biomasa [g/dm³]	% decrease % spadku	
			Culture turbidity Mętność kultury	PV value Wartość TN
1	2.5	1.68	32.2	57.6
2	5.0	2.16	43.5	56.3
3	10.0	2.62	43.5	60.6
4	20.0	2.74	49.2	57.2
5	30.0	2.86	53.2	57.0
6	50.0	3.06	58.0	52.2
7	80.0	3.44	61.3	50.0

PME — as in Table 1
EKS — jak w Tab. 1
PV — permanganate value
TN — test nadmanganianowy

degree of PME purification from organic compounds, as indicated by turbidity and PV, is greater at lower light intensities. From this we may conclude that at high light intensities, growth takes place more as a

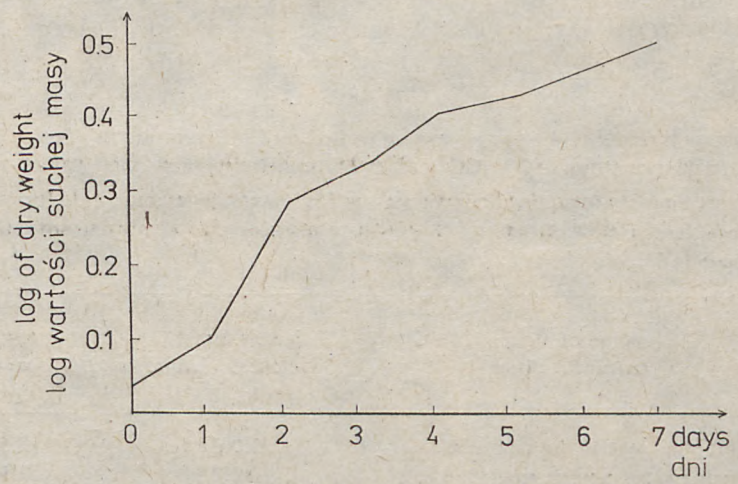

Fig. 1. Dynamics of growth of Chlorella pyrenoidosa No. 366 cultivated on PME solution in dilution 1 : 30, light intensity — 50 klx, inoculum value — 136 mg d.w./dm³

Rys. 1. Dynamika wzrostu kultury Chlorella pyrenoidosa nr 366 na EKS w rozcieńczeniu 1 : 30, przy oświetleniu 50 klx, wielkość inoculum 136 mg s.m./dm³

result of photosynthesis than because of the assimilation of organic substances from the medium. High light intensities thus give a high yield of Chlorella, but do not provide optimum conditions for efficient PME purification. Therefore, in later experiments, the light intensity was set at 50 klx, thus ensuring both a good yield of Chlorella and reasonable degree of PME purification. In further experiments, the growth dynamics of Chlorella pyrenoidosa were studied with respect to time, using the optimum light intensity. Fig. 1 shows that over a 7-day period, Chlorella grows continuously in 1 : 30 PME, although the most intensive growth takes place between the 2nd and 4th day.

However, the size of the inoculum also has a considerable influence on the growth rate. A preliminary experiment showed that starting with Chlorella cultures of 60 mg d.w./dm^3 and 360 mg d.w./dm^3, the biomass increase after 4 days was 2.0 and 3.5 g d.w./dm^3 respectively. Starting with an inoculum of 1 g, a biomass increase of up to 2.5 g d.w./dm^3 was obtained within 24h. So, in further experiments, the inocula used were as large as possible, while at the same time, the culture period was reduced to 24 h, one which could be implemented in purification plants.

3.4. Growth of other algae in PME

Four different species of algae — Chlorella pyrenoidosa No. 366, Scenedesmus acutus No. 449, Spirulina platensis and Chlorella sp. var. halofila — were grown under the optimum conditions described above, i.e. a 1 : 30 PME dilution, a light intensity of 50 klx, continuous aeration with an air +5% CO_2 mixture, a 24 h growth period and a large inoculum (around 1 g d.w./dm^3).

Table 6. Biomass increase of 4 algae species cultivated on PME solution in dilution 1 : 30 (light intensity — 50 klx, aeration +5% CO_2) after 24h cultivation with high values of inoculum

Tab. 6. Przyrost biomasy 4 glonów hodowanych na EKS w rozcieńczeniu 1 : 30 (przy oświetleniu 50 klx, przewietrzaniu powietrzem +5% CO_2) w czasie 24 godzin i przy stosowaniu wysokich wartości inoculum

No. Nr	Kind of algae Gatunek glonu	Inoculum [g d.w./dm^3] Wartość inoculum [g s.m./dm^3]	Biomass [g d.w./dm^3] Biomasa [g s.m./dm^3]
1	Chlorella pyrenoidosa nr 366	1.0068	2.5714
2	Scenedesmus acutus nr 449	0.9556	2.1628
3	Spirulina platensis	0.7228	2.6608
4	Chlorella sp. var. halofila	1.0267	2.0294

PME — as in Table 1
EKS — jak w Tab. 1

Since the algae Spirulina and Chlorella sp. var. halofila are halophytes [3, 4, 7], salts were added to the medium so that, together with the salts in the extract, the total salinity was 16.9 g/dm³. The weight of mineral components in the PME was 1.8 g/dm³, so 15.1 g/dm³ of salts were added (2.2 g/dm³ $NaHCO_3$ and 12.9 g/dm³ NaCl).

The concentration of the basic elements was similar to that in the

Table 7. Uptake of nutrients and depletion of PV and BOD values in 24h cultures of 4 algae species cultivated on PME solution (1 : 30). Light intensity – 50 klx, aeration +5% CO_2, high inoculum levels

Tab. 7. Zużycie pierwiastków biogennych i spadek wartości TN i BZT_5 po 24-godzinnej hodowli 4 glonów na EKS w rozcieńczeniu 1 : 30, oświetleniu 50 klx, przewietrzaniu powietrzem +5%CO_2 i użyciu wysokich inoculatów

No. Nr	Kind of algae Gatunek glonu	% loss % ubytku						
		N	P	Mg	S	Fe	PV TN	BOD BZT_5
1	Chlorella 366	58.2	46.2	63.3	23.5	64.4	40.3	95.6
2	Scenedesmus 449	63.3	42.7	40.0	17.2	80.4	38.6	91.3
3	Spirulina pl.	58.2	52.9	49.3	66.3	73.8	51.1	96.5
4	Chl. halofila	66.6	43.6	47.3	43.8	74.6	50.4	93.0

PME – as in Table 1
EKS – jak w Tab. 1
BOD – biochemical oxygen demand
BZT_5 – biochemiczne zapotrzebowanie na tlen
PV – permanganate value
TN – test nadmanganianowy.

experiments already carried out with 1 : 30 PME (Table 1). Cultures of halophytic organisms need a high sodium concentration for normal growth. Table 6 gives the increase in d.w. of 24 h cultures of the four algae. Spirulina platensis and Chlorella pyrenoidosa gave the highest yields. The use of large inocula (from 0.7 to 1.0 g d.w./dm³), together with a high light intensity pays good dividends, as the biomass increases in a 24h culture exceed those obtained in a 14-day culture of Chlorella pyrenoidosa (Table 2); N.B., in the latter, inocula of around 120 mg d.w./dm³ were used.

The extent of PME purification during algal culture was indicated by the percentage loss of biogenic elements and the decrease in PV and BOD (Table 7).

It can be seen from these data that nitrogen loss was around 60% (80% in the 14-day culture), phosphorus from 40 do 50% (30 - 40%), magnesium from 40 to 60% (40 - 50%). The uptake of sulphur and iron is significantly higher in the 24 h culture. The decreases in PV and BOD are similar order.

In conclusion, it can be stated that all the algae tested are suitable for use in the biological purification of waste water from pig farms. The halophytes, while purifying the PME as efficiently as the other algae, do require the addition of large quantities of NaCl to the medium, which may, in practice, become troublesome and render the process unprofitable. On the other hand, Chlorella pyrenoidosa can be recommended without reservation as an organisms which efficiently purifies waste water with a heavy load of organic matter. On a large scale, optimum growth conditions for this organism must be provided if both efficient waste water purification and large quantities of a valuable feedstuff are to be obtained.

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LABORATORYJNA OCENA PRZYDATNOŚCI GLONÓW W PROCESACH OCZYSZCZANIA ŚCIEKÓW Z DUŻYM OBCIĄŻENIEM MATERIĄ ORGANICZNĄ

Streszczenie

Przebadano wzrost miksotroficznego glonu Chlorella pyrenoidosa na ekstraktach z kału świńskiego. Są one bogate we wszystkie biogenne pierwiastki i zapewniają dobry wzrost tego glonu, ponad dwukrotnie wyższy w porównaniu z kontrolą mineralną.

W czasie wzrostu kultur glonów dochodzi do znacznego zużycia związków zawartych w ekstraktach, np. N do 87%, P do 44%, Mg do 52%, S do 51%. Spada również mętność ekstraktów.

Spadek zawartości substancji organicznej w trakcie hodowli glonów na ekstraktach kału jest znaczny. Np. wartość testu nadmanganianowego obniża się po hodowli o 69%, a wartość BZT_5 spada o 95%, co wskazuje, że badany glon jest organizmem przydatnym do oczyszczania tego typu ścieków.

Przeprowadzono optymalizację wzrostu tego glonu na ekstraktach z kału świńskiego. Jako optymalne parametry ustalono: stężenie ekstraktów 1 : 30, natężenie światła 50 klx, nieprzerwane przewietrzanie z dodatkiem 5% CO_2, wysokie inoculum i czas hodowli 24 h. W tych warunkach przeprowadzono również hodowlę innych glonów: Scenedesmus acutus, Spirulina platensis i Chlorella sp. var. halofila. Również te glony dają dobry przyrost biomasy na ekstraktach z kału świńskiego, a stopień oczyszczania podłoży towarzyszący ich wzrostowi jest znaczny.

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