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AN ATTEMPT OF APPLICATION OF ENZYMES TO WASTEWATER TREATMENT

An attempt has been made to apply purified enzymes (amylase and proteinase), isolated from activated sludge, to intensify the wastewater treatment. It has been stated that the addition of enzymes to activated sludge shortens remarkably the decomposition time of such substances as carbohydrates and proteins.

1. INTRODUCTION

In the recent times many communications have appeared, which indicated the increasing applicability of enzymes in the technology of wastewater treatment. OVERBECK et al. [11] and TEUBER [13] have shown that the degradation of organic compounds present in wastewater may be considerably accelerated by addition of isolated enzymatic preparations. LEVIEAU [8] has stated that the introduction of α- and β-amylases into the activated sludge accelerated substantially the degradation of starch contained in food industry wastewater. MUNNECKE [10] used a system of immobilized enzymes to remove the residual pesticides. Enzymatic decomposition — according to these authors — is particularly advantageous for the following reasons:

the specifity of enzymatic action may — in the case of pesticides — include a whole group of compounds or single substances,

enzymatic reactions are much faster than the chemical ones,

no additional chemical substances which may contaminate environment are introduced.

MUNNECKE has isolated hydrolyzing enzyme "parathion" and a whole group (8–12) of other phosphateorganic insectides. Decomposition of phosphate organic pesticides in presence of this enzyme proceeds much faster than in all the so far known chemical reactions. This enzyme is, moreover, characterized by a high stability and resistance to the action of chemical solvents. The "parathion-hydrolase" enzyme was covalently bound on cellulosis or glass. Preliminary results have shown:

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high stability of the enzyme — after a continuous hydrolysis lasting for two weeks it lost only 10% of its initial activity,

degradability of pesticides from 42% to 97%, depending on the flow rate accross the column and concentration of pesticides in solution,

immobilized enzymes may control the remnants of pesticides as well as in medicine (in therapy of poisonings with pesticides).

In the recent times the scientists all over the world have shown a great interest in utilization of hardly-degradable pollutants [1]. Since many years an enzymatic preparation "drain cleaner" used to liquidy the clogs in sewers has been produced by Miles Chemical Co. This preparation, apart from aerobic and anaerobic bacteria, contains also three enzymes: amylase, protease and lipase. It is applied in form of a water solution [2].

In the technological processes, presented briefly above, the enzymes after having performed their role of biological catalysts may be subject to thermal or chemical inactivation. At present, more attention is given to the production of immobilized enzymatic preparations.

A new "production technology of immobilized enzymes" in the nearest future may substantially contribute to the change in the technology of their application. The so far used static system, in which the enzyme is inactivated directly, may be replaced by a dynamic continuous sytem, in which the substrate is introduced either to reaction tank with immobilized enzymatic preparations or to columns filled with these preparations.

In both the cases, the products of enzymatic reaction can be easily separated from the enzymatic preparation. Clearly, such a method of enzymatic processing limited by the form of substrate, may be used in liquid media only.

The shortcoming of the above method is that the enzyme, after its reuse, may be partially or even totally inactivated.

The research on the methods of isolation of some enzymes from activated sludge has been conducted in the Institute of the Environment Development in Wrocław since many years. The so far developed method allowed us to separate two enzymes: α-amylase and proteinase in a crystalline form [5, 6]. As it is well known α-amylase catalyzes hydrolytic decomposition of polysaccharides to monosaccharides, while proteinase catalyzes the hydrolysis of proteins to amino acids.

The purpose of the present investigations was to use enzymes (α-amylase and proteinase) isolated from activated sludge to intensify the treatment of wastewaters coming from food industry.

The investigations included: biochemical analyses combined with physicochemical and technological tests of municipal wastewater and food industry wastewater.

2. MATERIAL AND METHODS

Activated sludge from wastewater treatment plant in Ciernie (district Wałbrzych) was used to isolate the enzymes the activities of which were tested.

Wastewater used for investigations came from the treatment plant of Namysłów potato industry.

The sums of mono- and bisaccharides were determined quantitatively by the method with anthron according to HEWITT [4]. Proteins were determined quantitatively by the method according to LOWRY [9]. Quantitative content of organic carbon was determined in the apparatus of the Beckman Model 515 H. Total amount of organic carbon was calculated from the difference between the amounts of total carbon and inorganic carbon. The remaining indices of pollutions occurring in wastewater were determined according to Polish Standards or by the methods given by HERMANOWICZ [3]. Amylase activity was determined by measuring the increment of sum of saccharides released during incubation of enzyme with the substrate (starch). The activity was expressed in micromoles of glucose. The incubated mixture was composed of 1.0 cm^3 of 0.1 M phosphate buffer (pH 7), 0.01 M $Ca(C_2H_3O_2)_2$, 0.25 cm^3 of enzyme containing 50 μg of enzymatic protein, and 10 mg of starch. This mixture completed to the final volume of 2.0 cm^3 was incubated at 37°C for about 1 h. Enzymatic reaction was interrupted by addition of two volumes of 96% ethanol. Eventually enzyme was denaturized and the excess of non-decomposed substrate was precipitated. The quantity of amylase enzyme, which under given conditions releases 1 micromol of sugar, has been assumed as a unit of activity. Specific activity has been expressed by the quantity of released glucose with respect to 1 mg of enzymatic protein. Proteins were determined by the LOWRY method [9]. Activity of protease was determined according to the modified method of ANON [7], assuming that the unit of activity is the quantity of this enzyme releasing during 1 min 1 micromol of tyrosyne from denaturated hemoglobine, counted for 1 mg of enzymatic protein. Incubation comprised mixture 1.0 cm^3 of 0.05 M phosphate buffer (pH 7.0), 2.0 cm^3 of enzymatic solution containing 0.5 mg of protein and 2.0 cm^3 of substrate (hemoglobine) solution. The mixture was incubated at 50°C for 30 min. Enzymatic reaction was stopped by adding trichloroacetic acid until its final (3%) concentration was reached. Eventually enzyme and excess of substrate not decomposed are precipitated. The samples were left for 10 min and then centrifugated. The precipitate (deposit) was removed and supernatant taken for determining tyrosine content by means of Folin's reagent. The determined quantity was referred to 1 mg of enzymatic protein.

The experiments were conducted in two parallel series, using an open system which consisted of 6 opened reaction tanks containing 1 dm^3 of suspended microorganisms of activated sludge. In the model installation the aeration was performed with compressed air, taken from a bottle and let through a filter and surge tank, the pressure being continuosly registered. A uniform distribution of the air in aeration tanks was ensured by metal grates which were suspended above the bottoms of tanks. The quantity of air introduced was dosed by means of rotameters.

Wastewater after mechanical treatment and containing high amounts of starch came from the treatment plant of Namysłów potato industry. In experiments with enzymes the following preparations were used: crystalline α-amylase, isolated from the activated sludge by the authors' own method [5], of the activity amounting to 4 micromols of glucose/mg of protein/min and crystalline protease isolated also from activated sludge [6], its specific activity was equal to 2.12 proteolytic units /mg of protein/min. Wastewater used in experiment contained 10 g of starch/dm^3 and 100 mg of protein/dm^3.

Enzymes were added to each sample of the series examined, maintaining their adequate ratio (expressed in enzymatic units determined earlier) to the wastewater volume used for investigations. Activated sludge, obtained from primary treatment plant in Ciernie, district Wałbrzych, was characterized by the following parameters: dry weight ranged within 2,000–3,000 mg/dm^3, whereas dry organic weight was equal to 81.5%. Index ranged within 60–100 cm^3/g. The experiment was conducted in two parallel series, applying different loading of activated sludge.

The first series consisted of 3 different mixtures and was a control series:

A) 1 dm^3 of activated sludge with the addition of 0.5 dm^3 of wastewater,

B) 1 dm^3 of activated sludge with the addition of 2.0 dm^3 of wastewater,

C) 1 dm^3 of activated sludge with the addition of 8.0 dm^3 of wastewater.

The second series, besides activated sludge and wastewater, contained also enzymes and constituted experimental series.

3. RESULTS

In wastewaters mechanically treated in the Namysłów Factory of Potato Industry a full analysis of the selected physicochemical indices have been made. Since in the separate aeration chambers the volumes of wastewater during measurements were different, only those indices were analysed which significantly determined the changes occurring in the removal of pollutants present in the wastewater examined. The experiments were conducted for 24 h period, samplings being taken after 2 h, 6 h, 12 h, and 24 h of aeration. While taking the samples, aeration was stopped for about 30 min. Afterwards from each aeration tank about 50 cm^3 of supernatant was taken to analyse the carbohydrates, organic nitrogen and organic carbon. Variability of the pollution indices during aeration of wastewaters in the presence of activated sludge and with or without enzymes is presented in table and figs. 1–3. From the data presented it follows that the addition of enzymes significantly influences the removal of pollutants present in wastewater examined. The effect of amylase on biodegradation of carbohydrates may be explained by the fact that in enzymatic hydrolysis the decomposition of starch to glucose proceeds much faster. The molecule of glucose is next up taken by microorganisms of activated sludge and, after a series of glycolytic reactions, is finally decomposed into CO_2 and H_2O in the cycle of tricarboxylic acids. From the investigations it follows also that the activated sludge used in experiments had no exoenzymes. These hypotheses were confirmed by the data presented in fig. 1 from which it follows that introduction of amylase into reaction chamber containing activated sludge and wastewater causes a distinct degradation of starch present in wastewater, its initial amount of 800 mg/dm^3 being reduced to 100 mg/dm^3. Thus, the removal of starch equals 87.5%, whereas in the absence of enzyme it is as low as 32%. After a 24 h aeration the respective values are equal to 97.7% and 64%.

Since starch was the main source of carbon in the wastewater examined, it seemed advisable to register the changes in the organic carbon content while measuring the changes

in the contents of carbohydrates. As it follows from the data presented in fig. 2 quantitative changes in the content of organic carbon follow the same direction as that of carbohydrates.

Table

Selected indices characterizing the effects of α-amylase and proteinase on wastewater treatment
Wybrane wskaźniki charakteryzujące wpływ α-amylazy i proteinazy na oczyszczanie ścieków

Index	Unit	Raw wastewater	Wastewater + activated sludge without enzymes (control)	Wastewater + activarted sludge with enzyme (sample examined)	Removal percent	
					With respect to control sample	With respect to sample examined
Turbidity	mg SiO_2/dm^3	1800	300	112.5	83.34	93.75
BOD_5	mg O_2/dm^3	2900	1400	1000	51.73	65.52
COD	mg O_2/dm^3	6606	2000	502	69.73	92.41
Permanganate value	mg O_2/dm^3	2250	680	220	69.78	90.23
Chlorides	mg Cl/dm^3	200	140	99	30.00	55.50
Total iron	mg Fe/dm^3	5.2	2.8	2.0	46.16	61.54
Total phosphorus	mg PO_4/dm^3	22.3	15.6	18.4	30.05	17.49
Dry weight	mg/dm^3					
total amount		7445	2120	1282	71.53	82.79
volatile parts		5691	1066	718	81.27	87.39
mineral parts		1754	1054	184	40.00	89.51

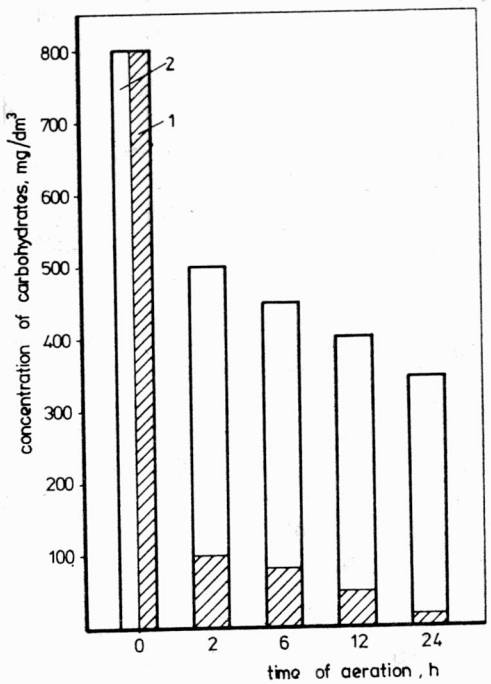

Fig. 1. Variations in carbohydrate contents during a 24 h aeration of the mixture of wastewater with activated sludge in the absence and presence of α-amylase

Rys. 1. Zmiany zawartości węglowodanów w czasie 24 h napowietrzania ścieków z osadem czynnym z udziałem i bez udziału α-amylazy

It seemed also of interest to state whether the effects observed in the changes of carbohydrate and organic carbon contents after activated sludge had been enriched with amylase will also appear with respect of proteins and total nitrogen due to the addition of a complex or proteolytic enzymes.

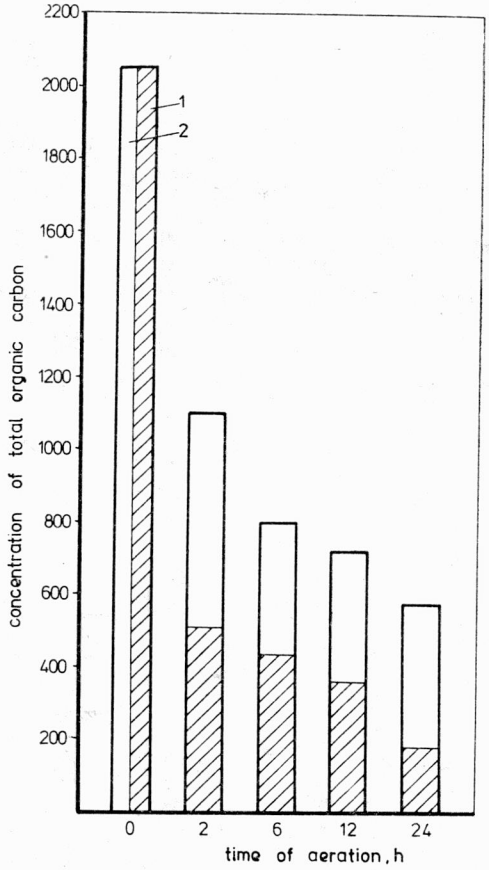

Fig. 2. Variations in organic carbon contents during a 24 h aeration of the mixture of wastewater with activated sludge in the absence and presence of α-amylase

Rys. 2. Zmiany zawartości węgla organicznego w czasie 24 h napowietrzania ścieków z osadem czynnym z udziałem i bez udziału α-amylazy

The experiments were performed identically as in the case of amylase (aeration time, the way and times of samplings). After 30 min sedimentation the total nitrogen was determined. The changes observed are illustrated in fig. 3. As it follows from the data presented the introduction of proteolytic enzymes caused a marked decrease of total nitrogen from the initial value of 400 mg/dm^3 to 86 mg/dm^3, which expressed in percent gives 78.5%

removal of nitrogen after 2 h aeration. After 24 h aeration under the same conditions the nitrogen removal reaches 96%. The obtained results prove that enzymes introduced additionally to the suspension of activated sludge and wastewater significantly accelerate biodegradation of proteins.

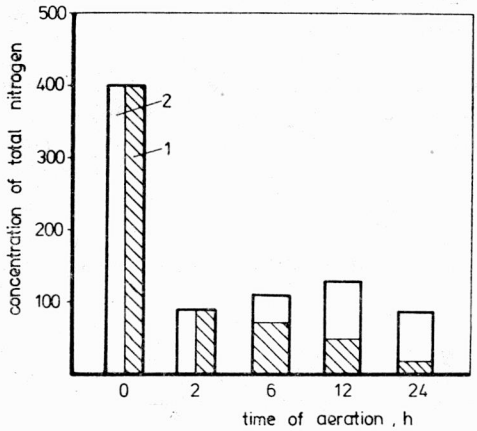

Fig. 3. Variations in total nitrogen contents during a 24 h aeration of the mixture of wastewater with activated sludge in the absence and presence of α-amylase

Rys. 3. Zmiany zawartości azotu ogólnego w czasie 24 h napowietrzania ścieków z osadem czynnym z udziałem i bez udziału α-amylazy

4. CONCLUSIONS

1. Wastewater from food industry and containing carbohydrates and proteins may by treated by the activated sludge method enriched with enzymes.

2. Introduction of enzymes to aeration tanks containing activated sludge and wastewater yields 87% removal of carbohydrates and proteins after 2 h aeration.

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PRÓBA ZASTOSOWANIA ENZYMÓW W OCZYSZCZANIU ŚCIEKÓW

Podjęto próbę zastosowania wydzielonych z osadu czynnego i oczyszczonych enzymów (amylazy i proteinazy) do intensyfikacji oczyszczania ścieków. Stwierdzono, że dodatek enzymów do osadu czynnego w dużym stopniu skraca czas rozkładu węglowodanów i białek.

EINSATZVERSUCH VON ENZYMEN IN DER ABWASSERREINIGUNG

Es wurden Proben vorgenommen, die aus dem Belebtschlamm abgesonderten und gereinigten Enzyme (Amylase und Proteinase) zur Intensivierung des Abwasserreinigungsvorganges wieder einzusetzen. Ein Zusatz der genannten Enzyme beschleunigte den Abbau von Kohlehydraten und Einweißstoffen mittels Belebtschlamm.

ПОПЫТКА ПРИМЕНЕНИЯ ЭНЗИМОВ В ОЧИСТКЕ СТОЧНЫХ ВОД

Предпринята попытка применения выделенных из активного ила и очищенных энзимов (амилазы, протеиназы) для интенсификации очистки сточных вод. Отмечено, что добавка энзимов к активному илу в большой степени сокращает время разложения углеводов и белков.