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Assessment of Metabolic Activity of Single and Mixed Microorganism Population Assigned for Potato Wastewater Biodegradation

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Abstract

An attempt was made to characterise thermophilic microflora isolated in an industrial plant utilising food industry waste. Initial estimation of environmental requirements of a mixed microorganism population was performed using the method measuring changes in electrical impedance by means of the BacTrac 4100 Automatic Microorganisms Growth Analyzer. Control of the dynamics of microorganism growth on broth media supplemented with sources of carbon, nitrogen and phosphorus and at various pH allowed initial assessment of their environmental requirements. The course of alterations in electrical impedance during culturing of both single isolates and mixed microorganism populations can be described by means of Gompertz's curve characteristic for bacteria growth with a very high correlation coefficient. The mixed microorganism population and isolates obtained from it were also characterised from the point of view of their biodegradation potentials of wastewater obtained in the potato processing industry. Biodegradation processes carried out in conditions of shake flask cultures at a temperature of 55°C showed higher reducing potentials of chemical oxygen demand (COD) of a mixed bacteria culture. Single isolates were characterised by a distinctly inferior metabolic activity and lower biodegradation potentials of " hot" potato wastewater.

Keywords: mixed microorganism population, metabolic activity, electric impedance, wastewater, thermophilic biodegradation

Introduction

In connection with Poland's accession to the European Union, it will soon become necessary to comply with the directive of the EU Commission concerning environmental protection. This is associated with the need to find new technologies for biodegradation and cleaning of wastes and sewage from agro-industry as well as their stabilisation and hygienisation [7, 16]. Wastewater from food industry is usually characterised by a high biological load and considerable problems with its biodegradation. In addition, part of it is characterised by temperatures higher than those utilised in mesophilic biological wastewater treatment technologies [6].

Wastewater from enterprises manufacturing potato starch is characterised by very high indices of biological oxygen demand - BOD_5 (of up to 26 g O_2/L) and chemical oxygen demand - COD (up to 77 g O_2/L) [8]. Since part of this wastewater, e.g. that derived from the process of acid-thermal coagulation (deproteinization) of potato juices, is characterised by increased temperature, there are possibilities of its biodegradation using thermophilic microorganisms [12, 13].

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Thermophilic systems of aerobic biological wastewater treatment have been known since the 1950s, while in the past five years, because of a dramatic increase of interest in these systems, first constructions of these processes have been developed. Despite the fact that thermophilic aerobic biological wastewater treatment has many advantages, physical, chemical and biological characteristics of this process are so different from mesophilic activated sludge process that our knowledge about how to manage the latter systems is of little help in the case of thermophilic processes [9,15]. In contrast to other methods of utilisation of wastewater from food industry and food wastes in general, thermophilic aerobic biological wastewater treatment is little known with regard to microbiological changes taking place in the course of fermentation as well as to the type of microflora taking part in processes of thermophilic biodegradation of wastewater components [2, 10, 18].

Thermophilic aerobic biological wastewater treatment is characterised by a higher (in comparison with other processes) substrate degradation, rapid inactivation of pathogenic microorganisms and low production of activated sludge [3]. High biodegradation rates shorten process time (detention time in bioreactor) and, consequently, reduces investment expenditure required for a wastewater treatment plant. Moreover, high biodegradation rates may also improve stability of the entire processes due to rapid solutions of critical situations [17]. Therefore, thermophilic aerobic systems appear to be particularly suitable and useful for the utilisation of highly concentrated wastewater with low flow intensity, dangerous wastewater (with pathogens) as well as wastewater characterised by high salinity levels. Disadvantages of the aerobic thermophilic process are associated with considerable costs of aeration of bioreactors, low capabilities of thermophilic microflora for flocculation and problems with foaming during fermentation [9, 13, 15].

Attempts to apply thermophilic aerobic biological treatment of wastewater from the potato processing industry were made by Malladi and Ingham [12]. Wastes for breweries were investigated by Zvauya et al. [21]. Studies were also conducted to apply this method to the utilisation of molasses [11], wastewater from slaughterhouses [4] and from the dairy industry [7]. Very little information is available concerning diversity of microflora composition in the course of the thermophilic aerobic process of wastewater treatment. Only a few researchers attempted to isolate cultures from bioreactors in which thermophilic aerobic wastewater treatment was taking place [18]. Most commonly, the isolated organisms belonged to bacteria from the genus *Bacillus* [1, 19].

Authors of this project made an attempt to characterise the activity of thermophilic microflora isolated in an industrial plant utilising the wastes from food industry. The mixed culture of microorganisms and isolates obtained from it, were characterised from the point of view of dynamics of their metabolic activity and biodegradation capabilities of wastewater from a potato processing plant.

Materials and Methods

Material

The material for investigations was wastewater of potato juices after deproteinization by the acid-thermal coagulation method (so called "hot" wastewater) from Wielkopolskie Przedsiębiorstwo Przemysłu Ziemniaczanego in Luboń. The material was collected during the 2000 autumn production campaign. The temperature of the collected wastewater ranged from 80-85^{o}C, while its chemical oxygen demand ranged from 21.5 to 28 g O_2/L.

Microbiological Media

Most of the cultures in which the BacTrac 4100 analyzer was applied were carried out using nutrient broth medium (BTL Łódź). In addition, a special medium for determining total number of microorganisms, employing the impedance changes measurement method called 001A, was also used.

Microorganisms

Investigations were carried out using thermophilic mixed microbial population isolated in an industrial plant utilising wastes from food industry and on single isolates obtained from it. The culture of mixed microorganisms, of which a majority were bacteria of the genus *Bacillus*, was adopted to growth on "hot" potato wastewater. The culture was maintained through regular passaging (every three days) onto a sterile wastewater substrate of pH = 7.0 (shake flask culture, temp. 55^{o}C, 150 rpm). The 48 h culture prepared in this way was used for culture inoculation. Single isolates were stored on agar slants (Plate Count Agar – Merck) at 4^{o}C.

Methods

Estimation of Metabolic Activity Using the BacTrac 4100 Automatic Microorganisms Growth Analyzer

Assessment of the metabolic activity of tested microorganisms was performed employing the method of electrical impedance changes measurement using the BacTrac 4100 Automatic Microorganism Growth Analyzer (SY-LAB, Austria). The measurement principle consists in a continual registration of changes in electrical impedance caused by metabolic processes of microorganisms. Molecules with high molecular weight (e.g. proteins, carbohydrates) forming part of the culture medium are broken down into ions in the course of metabolic processes of microorganisms. Smaller molecules increase the conduction of electrical current and, consequently, reduce environmental impedance. The tested microorganisms are incubated in special measuring cells equipped with four electrodes. Every 10 minutes changes are registered, simultaneously and independently of one another, in the impedance directly in the culture medium (changes in environmental conductivity) as well as on the surface of electrodes (changes in the electrode potential caused by ion adsorption on their surface). Impedance changes are expressed as percentages of changes in relation to initial values. A graphic picture of the correlation of these changes in time is a curve, which corresponds to the microorganism growth curve [5].

The measurement of impedance changes was carried out at 55°C in measuring cells of the 4100 BacTrac apparatus. 9 ml of culture medium (001A or wastewater) was placed in each cell and inoculated with 1 ml inoculum. Statistical analysis of results involved the description of electrical impedance changes in the course of culturing on the basis of a mathematical model characterising the bacterial growth curve. A comparison of metabolic activity of the mixed bacteria population during its growth on examined media was carried out employing Gompertz's tri-parametrical, dual logarithmic curve described by the following formula [22]:

$$y = a \cdot (-\exp(b - cx)) \quad (1)$$

All results subjected to statistical analysis are means from five replicates.

Wastewater Biodegradation in Conditions of Shake Flask Culture

Cultures were conducted in conditions of shake flask cultures (150 rpm) for 72 hours at 55°C on sterile wastewater, the initial pH of which was brought to 7.0 using 5M NaOH. The applied inoculum constituted 10% of the medium volume.

Throughout the entire culturing process, the following parameters were controlled: changes in medium pH (by potentiometric method), changes in the content of reducing substances (using the method with 3,5-DNS acid [14]), and changes in COD index (by colorimetric method using the Dr Lange cuvette test).

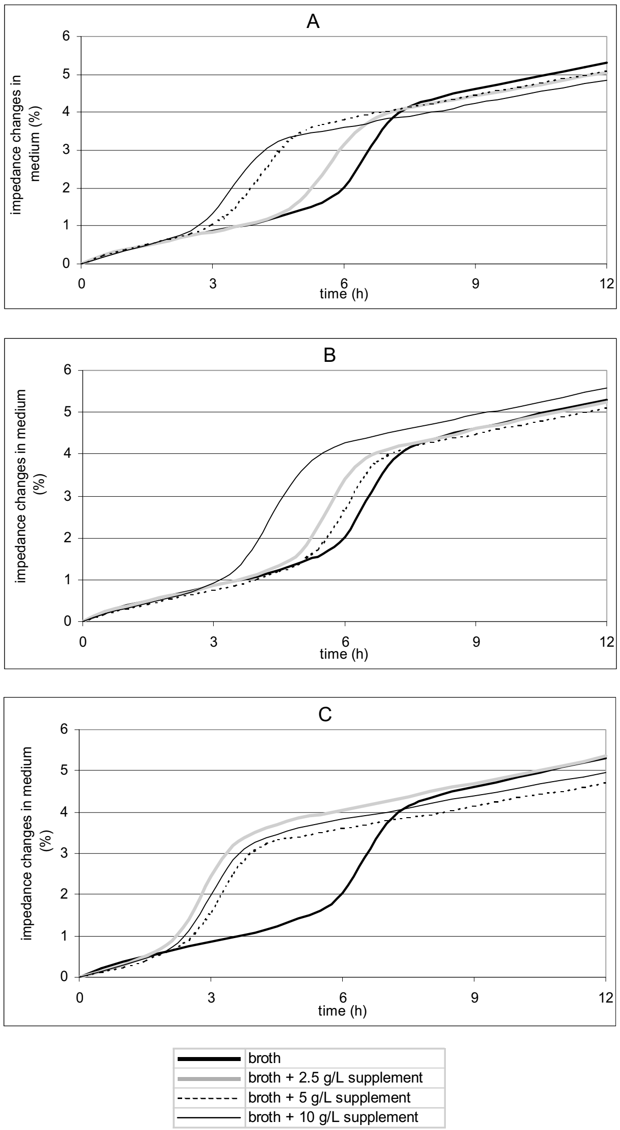

Fig. 1. Changes in electrical impedance of medium enriched with 2.5 to 10 g/L glucose (A), maltose (B) and soluble starch (C) during growth of mixed bacteria population.

Results and Discussion

Metabolic activity of the mixed bacteria population was assessed using the BacTrac 4100 Automatic Microorganisms Growth Analyzer. Cultures were conducted on broth media enriched with additional sources of carbon, nitrogen and phosphorus and at different initial pH of culture media.

The enrichment of media with additional sources of carbon: glucose, maltose and soluble starch, resulted in an increased metabolic activity of mixed bacteria population. The growth on media supplemented with 2.5 – 10 g/L carbohydrates was characterised by shortening of the lag-phase – the higher the supplementation with sugar, the shorter the period with no changes in electrical impedance (Fig. 1).

Curves of changes in medium electrical impedance in the course of culturing were described mathematically by Gompertz's model, which characterises the bacterial growth curve [22]. The matching of experimental curves, which were means from five repetitions, to Gompertz's mathematical model described by the formula (1) was characterised by a very high correlation coefficient $0.9869 < r < 0.9948$ ($\alpha \geq 0.001$) (Tab. 1). In addition, the maximum growth rates of mixed bacteria population on tested media and the points of curve inflection, indicating the time when the maximum growth rate was achieved, were also determined.

The analysis of bacterial mixed population metabolic activity on media enriched with glucose, maltose and soluble starch revealed a positive influence of the additional carbon source in experimental media on microorganism activity. Increased quantities of carbohydrates in the media resulted in a gradual shortening of the time necessary to achieve maximum growth rates (time to reach

Table 1. Changes in electrical impedance (BacTrac 4100) of media supplemented with selected saccharides during growth of mixed bacterial population described by Gompertz's mathematical model: y = a · (-exp(b-cx)).

Medium	Model's parameters			Maximum growth rate (i.c.u.* / h)	Inflection point (h)	Correlation of the model to experimental curves (r)
	a	b	c			
Broth	6.18	1.84	0.33	0.75	5.58	0.9869**
Broth + 2.5 g/l glucose	5.29	1.99	0.42	0.82	4.73	0.9906**
Broth + 5 g/l glucose	4.88	1.81	0.52	0.93	3.49	0.9937**
Broth + 10 g/l glucose	4.53	1.66	0.54	0.90	3.07	0.9921**
Broth + 2.5 g/l maltose	5.36	2.22	0.44	0.87	5.04	0.9898**
Broth + 5 g/l maltose	5.42	2.08	0.44	0.88	4.71	0.9904**
Broth + 10 g/l maltose	5.33	2.25	0.59	1.16	3.79	0.9948**
Broth + 2.5 g/l soluble starch	4.32	1.88	0.66	1.05	2.86	0.9906**
Broth + 5 g/l soluble starch	4.55	1.78	0.65	1.08	2.74	0.9911**
Broth + 10 g/l soluble starch	4.87	1.66	0.63	1.13	2.63	0.9893**

* i.c.u – impedance changes unit
** statistic significance (α≥0.001)

Table 2. Changes in electrical impedance (BacTrac 4100) of media of different pH during growth of mixed bacterial population described by Gompertz's mathematical model: y = a · (-exp(b-cx)).

Medium	Model's parameters			Maximum growth rate (i.c.u.* / h)	Inflection point (h)	Correlation of the model to experimental curves (r)
	a	b	c			
pH=6.0	3.57	1.46	0.41	0.54	3.53	0.9992**
pH=7.0	4.44	1.42	0.47	0.77	2.99	0.9970**
pH=8.0	4.09	1.76	0.42	0.64	4.17	0.9954**

* i.c.u – impedance changes unit
** statistic significance (α≥0.001)

Table 3. Changes in electrical impedance (BacTrac 4100) of media supplemented with $(NH_4)_2HPO_4$ during growth of mixed bacterial population described by Gompertz's mathematical model: y = a · (-exp(b-cx)).

Medium	Model's parameters			Maximum growth rate (i.c.u.* / h)	Inflection point (h)	Correlation of the model to experimental curves (r)
	a	b	c			
Broth	4.29	1.51	0.30	0.47	5.00	0.9967**
Broth + 0.5 g/l $(NH_4)_2HPO_4$	3.92	1.44	0.29	0.42	4.86	0.9968**
Broth + 1 g/l $(NH_4)_2HPO_4$	3.37	1.35	0.33	0.41	4.07	0.9952**
Broth + 2 g/l $(NH_4)_2HPO_4$	4.50	1.25	0.24	0.40	5.17	0.9989**
Broth + 5 g/l $(NH_4)_2HPO_4$	4.54	1.46	0.31	0.51	4.77	0.9958**

* i.c.u – impedance changes unit
** statistic significance (α≥0.001)

the Gompertz's curve inflection point) (Tab. 1).

Exploration of optimal growth conditions of mixed bacteria population allowed determining pH value intervals ranging from 6.0 to 8.0. Detailed examination of growth dynamics indicated that at pH=7.0 the examined culture was characterised by maximum growth rate (0.77 i.c.u./h) and the shortest time needed to reach it (2.99 h) (Tab. 2). Supplementation of the broth medium with additional sources of nitrogen and phosphorus in the form of $(NH_4)_2HPO_4$ did not lead to distinct alterations in the metabolic activity of used mixed bacteria population. In this case, maximum growth rates ranged from 0.4 to 0.5 i.c.u./h (Tab. 3), while the course of curves was very similar for all applied media composition (Fig. 2).

The metabolic activity of the mixed culture of microorganisms as well as of single isolates derived from it, was also assessed during culturing on 001A medium and on potato wastewater (Fig. 3). A distinctly higher level of impedance changes (over 45%) was registered during growth of microorganisms on 001A medium. This was caused by a special medium composition which was specially prepared to obtain high changes in electrical impedance. In the case of culturing of microorganisms on both media, the most intensive changes were observed during growth of mixed culture. Single isolates showed lower metabolic activity in both cases. This could have been caused by synergistic interrelationships between microorganisms occurring in the mixed culture, which were missing in monocultures leading to reduced biological activity [20].

The examined microorganisms were also tested from the point of view of their "hot" potato wastewater biodegradation potentials. Using sterile wastewater with pH brought to 7.0, 72 h shake flask cultures at 55°C were carried out. The media pH was found to increase up to 9.6, both in the case of single isolates and mixed culture (Fig. 4). The course of media pH changes was similar for all the applied microorganisms. The utilisation of reducing substances from wastewater was distinctly higher when mixed

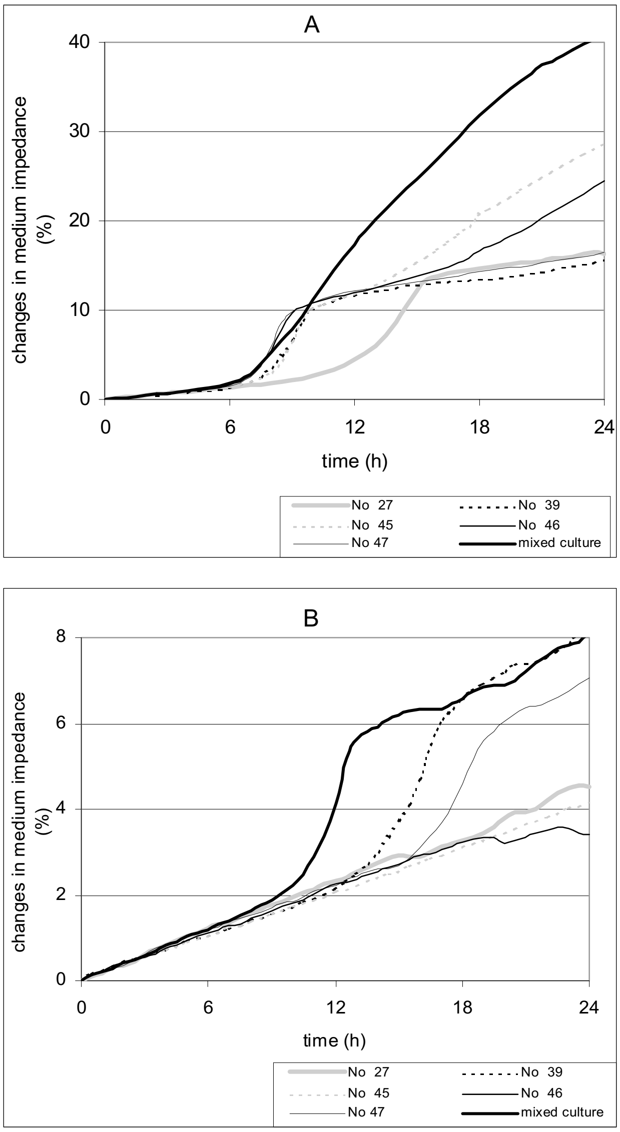

Fig. 3. Changes in electrical impedance of 001A medium (A) and potato wastewater (B) during growth of single isolates and mixed bacteria population.

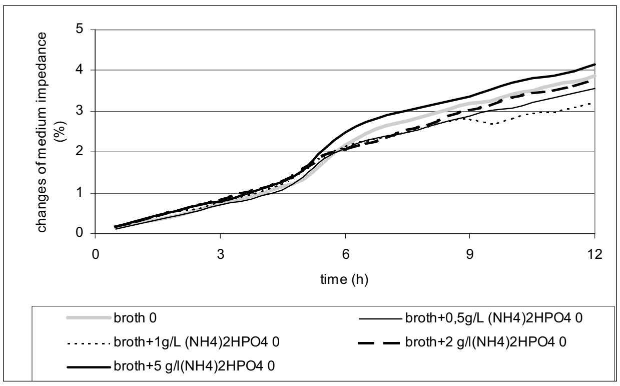

Fig. 2. Changes in electrical impedance of medium enriched with 1.0 to 5.0 g/L $(NH_4)_2HPO_4$ during growth of mixed bacteria population.

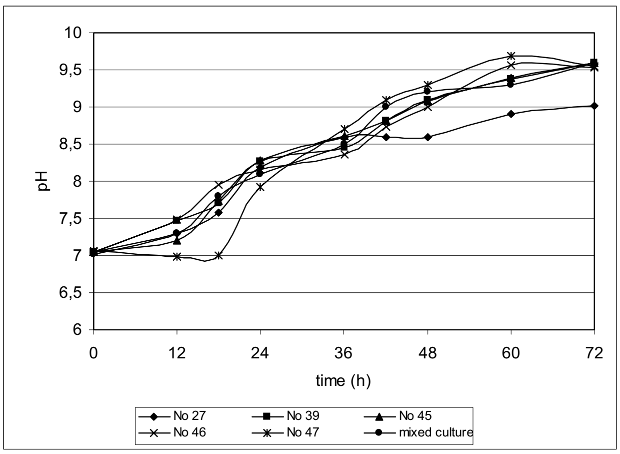

Fig. 4. Changes in pH of potato wastewater during fermentation by single isolates and mixed bacteria population.

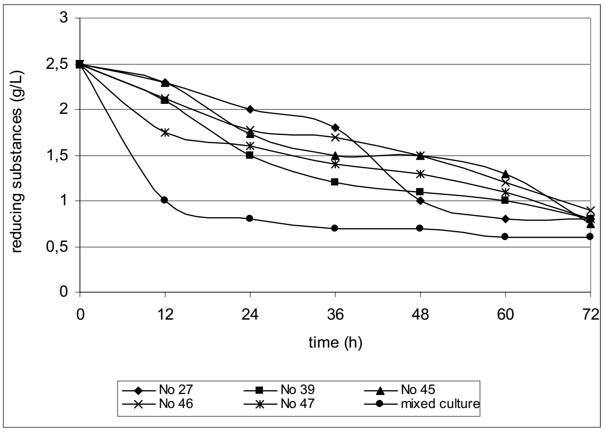

Fig. 5. Changes in the content of reducing substances in potato wastewater during fermentation by single isolates and mixed bacteria population.

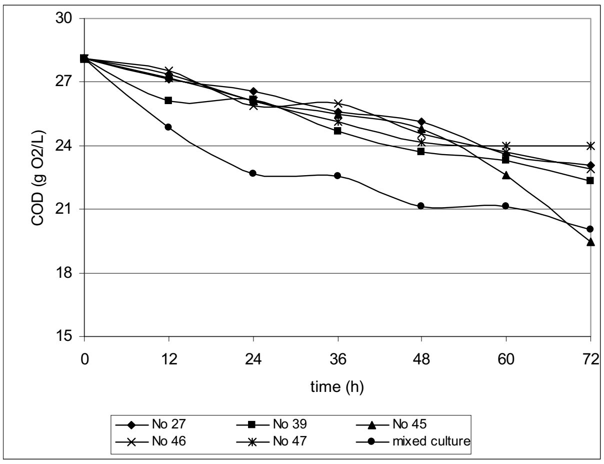

Fig. 6. Potato wastewater COD changes during fermentation by single isolates and mixed bacteria population.

thermophilic microorganism population was applied (Fig. 5). Similarly, it was also in a mixed microorganism culture that changes in wastewater COD during biodegradation were characterised by the highest reduction of the wastewater load index. The course of COD changes in the media for single isolates cultured under identical conditions was similar. None of the isolated strains showed distinctly better capabilities for wastewater biodegradation (Fig. 6). The decline of wastewater load expressed by the reduction of COD during shake flask culture did not exceed 31%. This was most probably associated with a high demand of thermophilic microflora for oxygen. When fermentation processes are performed in bioreactors with a possibility to aerate the culture medium intensively, this has a very positive influence on the reduction of COD, increasing in this way, the effectiveness of wastewater biodegradation [9]. Our initial investigations indicate that the application of a laboratory fermenter, characterised by good aeration possibilities, can significantly increase the extent of wastewater biodegradation obtained from a potato processing plant.

Conclusions

The results presented let us conclude that analysis of impedance changes caused by the growth of mixed bacteria population and single isolates permits assessing metabolic activity of microorganisms using different culture conditions. Changes in electrical impedance can be described by Gompertz's curve characteristic for bacteria growth with a very high correlation coefficient $0.9869 < r < 0.992$. The use of Gompertz's mathematical model to describe medium impedance changes in the course of culturing allowed determination of the maximum bacterial growth rate and the point of curve inflection, which indicates the time after which maximum growth rate is achieved.

The control of microorganism growth using the Bac-Trac 4100 Automatic Microorganism Growth Analyzer allows a rapid and accurate assessment of metabolic activity and initial optimisation of culture conditions. The majority of control methods estimating growth of microorganisms, e.g. microscopic inspection, traditional agar plates or nephelometric measurements, can only describe the quantitative increase of the number of microorganisms. For investigations of technological processes, the potential of microorganisms to perform a number of biochemical changes, i.e. their metabolic activity, is far more important than the growth rate.

Investigations carried out using shake flask culture indicated higher wastewater biodegradation capabilities of mixed bacteria population as compared to single isolates derived from it. Both, metabolic activity and COD reduction of wastewater, were distinctly higher for mixed bacteria population. Due to the possibility of the acceleration of the process (high temperature) and relatively low biomass production, the application of thermophilic microflora appears to be a good and perspective solution, especially for the initial treatment of "hot" wastewater with high biological load.

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References

1. BEAUDET R., GAGNON C., BISAILLON J.G., ISHAQUE M. Microbial aspect of aerobic thermophilic treatment of swine waste. Appl. Environ. Microbiol. **56** (4), 971, **1990**.
2. BOMIO M., SONNLEITNER B., FIECHTER A. Growth and biocatalytic activities of aerobic thermophilic populations in sewage sludge. Appl. Microbiol. Biotechnol., **32**, 356, **1989**.
3. CHEUNBARN T., PAGILLA K.R. Temperature and SRT effects on aerobic thermophilic sludge treatment. Jour. of Envir. Engin. **125** (7), 626, **1999**.
4. COUILLARD D., ZHU S. Thermophilic aerobic process for the treatment of slaughterhouse effluents with protein

recovery. Envir. Pollut. **79**, 121, **1993**.
5. FUTSCHIK K.,PFUTZNER H., DOBLANDER A., ASPERGER H. Automatical registration of microorganism growth by new impedance method. Abstr. Intern. Meetg. Chem. Eng & Biotechnol. Achema, **88**, 1, **1988**.
6. HASEGAWA S., SHIOTA N., KATSURA K., AKASHI A. Solubilization of organic sludge by thermophilic aerobic bacteria as a pretreatment for anaerobic digestion. Wat. Scien. and Technol. **41** (3), 163, **2000**.
7. KOSSEVA M.R., KENT C.A., LLOYD D.R. Thermophilic bioremediation of whey: effect of physico-chemical parameters on the efficiency of the process. Biotechn. Lett. **23**, 1675, **2001**.
8. KUTERA J., CZYŻYK F. Metody oczyszczania i utylizacji ścieków przemysłu rolno-spożywczego oraz odchodów zwierzęcych z ferm i obiektów inwentarskich. Wydawnictwo IMUZ, Warszawa, pp. 29-63 (in Polish), **1997**.
9. LAPARA T.M., ALLEMAN J.E. Thermophilic aerobic biological wastewater treatment. Wat. Res. **33** (4), 895, **1999**.
10. LIM B.R., HUANG X., HU H.Y., GOTO N., FUJIE K. Effects of temperature on biodegradation characteristics of organic pollutants and microbial community in a solid phase aerobic bioreactor treating high strength organic wastewater. Wat. Sci. and Technol. **43** (1), 131, **2001**.
11. LOLL U. Purification of concentrated organic wastewaters from the foodstuffs industry by means of aerobic-thermophilic degradation process. Progr. Wat. Technol. **48** (2/3), 373, **1976**.
12. MALLADI B., INGHAM S.C. Thermophilic aerobic treatment of potato-processing wastewater. World Journal of Microbiol. And Biotechnol. **9**, 45, **1993**.
13. MASON C.A., HANER A., HAMER G. Aerobic thermophilic waste sludge treatment. Wat. Sci. Tech. **25** (1), 113, **1992**.
14. MILLER G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar; Analytical chemistry , **31** (3) 426, **1959**.
15. PONTI C., SONNLEITNER B., FIECHTER A. Aerobic thermophilic treatment of sewage sludge at pilot plant scale. 2. Technical solutions and process design. Jour. of Biotech., **38**, 183, **1995**.
16. SKJELHAUGEN O.J. A Farmer-operated system for recycling organic wastes. J. Agric. Engng. Res. **73**, 373, **1999**.
17. SKJELHAUGEN O.J. Thermophilic aerobic reactor for processing organic liquid wastes. Wat. Res. **33** (7), 1593, **1999**
18. SONNLEITNER B., FIECHTER A. Bacterial diversity in thermophilic aerobic sewage sludge. Active biomass and its fluctuations. Eur. J. Appl. Microbiol. Biotechnol. **18**, 47, **1983**.
19. SURUCU G.A., ENGELBRECHT R.S., CHIAN E.S.K. Thermophilic microbiological treatment of high strength wastewaters with simultaneous recovery of single cell protein. Biotechn. and Bioeng. **17**, 1639, **1975**.
20. TYSZKA M., KASZYCKI P., KOŁOCZEK H., Metody współczesnej biotechnologii w procesach biodegradacji zanieczyszczeń. Ekologia i Technika. **6** (6), 175, **1998**.
21. ZVAUYA R., PARAWIRA W., MAWADZA C., Aspects of aerobic thermophilic treatment of Zimbabwean traditional opaque-beer brewery wastewater. Bioresource Technol., **48**, 273, **1994**.
22. ZWIETERING M. Modeling of the microbial quality of food, Praca doktorska, Landbouwuniversity, Wageningen., **1993**.