

Electrical impedance for bacterial metabolic activity screening-evaluation of single and mixed bacterial consortia for wastewater biodegradation

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Abstract: The use of an electrical impedance measurement method for selection of bacteria with high metabolic activity, specific for treatment of high loaded potato industry wastewaters was investigated. The dynamic and effectiveness of wastewater biodegradation were evaluated in two ways: by the analysis of electrical impedance changes caused by bacterial metabolism during bioremediation process as well as by the reduction of chemical oxygen demand (COD). The course of impedance signals were described with Gompertz mathematical model and a parameter of maximal impedance changes rate (*I_{max}*), expressed as % of impedance changes h⁻¹, was proposed for bacterial metabolic activity screening tests. A significantly higher ($p < 0.05$) metabolic activity of bacteria occurring in the mixed cultures in comparison with single isolates coming from them was determined. A linear correlation ($r = 0.89$), found between the wastewater COD reduction and the values of the maximal impedance changes rate (*I_{max}*) allowed the use of electrical impedance method and the proposed parameter for easier and quicker, in comparison with classic COD measurement, evaluation of the microbial wastewater treatment capability.

Keywords: Electrical impedance, single and mixed bacteria populations, potato processing wastewater, biodegradation

Introduction

Food industry produces one of the largest streams of industry wastewater. A high organic load, quite frequently campaign character (occurring temporarily in large quantities) of the wastewater, cause that despite many solutions there is still an urgent need to look for other new methods of rapid and effective ways of utilization. Most study is focused on the control of the process, modification of the technologies etc. On the other hand, little is known about the microflora operating in the processes. Microorganisms employed in the wastewater utilization include bacteria mainly from the genus *Bacillus*. They are used most commonly in systems of mixed populations (Sonnleitner and Fiechter, 1983; Surucu, 1999; Lapara *et al.*, 2001; Lim *et al.*, 2001; Cibis *et al.*, 2002; Krzywonos *et al.*, 2008). Mixed cultures taking part in wastewater biodegradation are formed generally in a natural way, in the result of their adaptation to environmental conditions as well as microbial synergistic and antagonistic interactions

(Surucu, 1999). The microorganisms occurring in mixed cultures are characterized by higher biological activity and smaller nutritive requirements in comparison with single isolates (Sakazawa *et al.*, 1981; Surucu, 1999; Lim *et al.*, 2001). Except that, in the case of processes carried out by use of mixed cultures there were observed higher product yields, higher growth rates and lower contamination dangers in confrontation with processes of single cultures (Yang *et al.*, 2003; He *et al.*, 2004).

A successful biodegradation process may require the use of multiple microorganism consortium with complementary metabolic capabilities. It can bring numerous potential benefits, including the ability to degradation of specific, toxic compounds and establishing catabolic pathways that have not been occurred by single cultures (Sakazawa, *et al.*, 1981; Shimao *et al.*, 1984; Ramos *et al.*, 1996; Oh and Bartha, 1997; Park *et al.*, 1999; Gilbert *et al.*, 2003).

The objective of the presented study was to select bacteria isolates characterized by high metabolic activity and capability for biodegradation and

afterwards to develop from them a mixed culture specific for the type of the treated wastewater. The selection of the isolates, characterized by high metabolic activity, was performed by employing the electrical impedance measurement method. The principle of the impedimetric technique is the measure of the electrical impedance changes caused by the metabolic processes of microorganisms in the culture medium. Molecules of high molecular weight present in the medium (e.g. proteins, carbohydrates and fats) are degraded during biochemical processes of microorganisms to ionized metabolic products. Smaller, charged molecules result in the increase of electric current conductivity and, consequently lead to the decrease in the electrical impedance of the culture environment (Noble, 1999; Gomez *et al.*, 2002; Yunus *et al.*, 2002). Primarily, this method is used for rapid detection or quality and quantity analyses of microorganisms, especially in the quality control of food, pharmaceutical and cosmetic articles (Wawerla *et al.*, 1999). However, attempts were made to employ impedance measurements for characterization of metabolic activities of microorganisms. Curda and Plockova (1995) investigated inhibiting properties of honey and its influence on the metabolic activities of lactic acid bacteria, while Paquet *et al.* (2000) applied the measurement of electrical impedance to assess the activity of starter cultures in the process of cheese production.

In this study, the electrical impedance method is proposed for selection of microorganisms consortia with high metabolic activity for biological wastewater utilization. The specific mixed bacteria cultures were developed for the biodegradation of high loaded wastewater from a potato processing plant.

Materials and Methods

Wastewater

Two types of wastewater obtained from potato processing plant (Wielkopolskie Przedsiębiorstwo Przemysłu Ziemniaczanego, Lubon, Poland) were used in the performed investigations: first, wastewater manufactured in the process of protein recovery from potato juices by means of acid-thermal coagulation – named hot wastewater, and second, wastewater created during the process of starch production – named starch wastewater (Table 1).

Microorganisms

Two thermophilic *Bacillus* mixed cultures were used in the performed investigations: *Mixed Culture C1* (*Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus laterosporus*, *Bacillus cereus*) isolated from

wastes derived from the fruit and vegetable industry (Lamm Resources Ltd., Corby, Northamptonshire, the United Kingdom) and *Mixed Culture C2* (*Bacillus laterosporus*, *Bacillus circulans*, *Bacillus fillocolonius*, *Bacillus acidocaldarius*, *Bacillus stearothermophilus*, *Bacillus licheniformis*) utilized for the process of hot potato slops biodegradation (Department of Bioprocess Engineering, Wrocław University of Economics, Poland). The individual isolates obtained from the two mixed cultures were identified by the Bergey key, API tests and the PCR method (unpublished data).

All the microorganisms, mixed cultures and single isolates coming from them were previously adapted to both types of wastewater, separately. The acclimatization was carried out by regularly passaging (every 3 days) on wastewater media by shake culturing (130 rpm) at 55°C (thermophilic conditions).

The inoculum was prepared using a nutritional broth (BTL, Poland) as a growth medium. The culture was carried out for 24 h in shaking conditions (130 rpm) at the temperature of 55 °C. The inoculum used for the inoculation constituted 10% (v/v) of the treated medium.

Evaluation of the metabolic activity of bacteria

The metabolic activity was performed by the method of electrical impedance changes measurement by using the Automatic Analyzer of Microorganisms Growth BacTrac 4100 (Sy-Lab, Austria). Special 10 ml measuring test tubes (Sy-Lab), equipped in 4 electrodes were used in the experiment. The test tubes were filled with 9 ml of wastewater, sterilized at 121°C for 15 min and then inoculated with 1 ml inoculum of the tested bacteria population. As the impedance changes strictly depend on the number of microorganisms in the sample, the inoculum was diluted to the range from $3 \cdot 10^3$ to $4 \cdot 10^3$ cfu ml⁻¹ and the number of viable bacteria cells in the inoculum was determined employing the classical plate method by using Plate Count Agar (Merck). The measuring test tubes were incubated in the thermostat of the Automatic Analyzer of Microorganisms Growth BacTrac 4100 for 24 h at 55°C. Changes in the electrical impedance of the treated wastewater, caused by metabolic processes of bacteria, were calculated in relation to the initial value according to the following formula:

Table 1. Chemical composition of potato starch wastewaters

Parameters	Unit	Concentration	
		Hot wastewater	Starch wastewater
Temperature	°C	80-85	10-20
pH	-	5.3 ± 0.2	5.6 ± 0.3
COD	[gO ₂ l ⁻¹]	31 ± 5	21 ± 5
BOD	[gO ₂ l ⁻¹]	18 ± 3	10 ± 3
Total organic carbon (TOC)	[g l ⁻¹]	9.4 ± 2.8	5.4 ± 1.2
Total nitrogen	[g l ⁻¹]	2.1 ± 0.3	1.2 ± 0.1
Total phosphate	[g l ⁻¹]	0.4 ± 0.1	0.2 ± 0.1

$$y = \frac{(y_0 - y_i)}{y_0} \cdot 100\%$$

y – changes in the electrical impedance of the wastewater
 y_0 – value of the electrical impedance at the beginning of culturing.
 y_i – value of the electrical impedance at a given point of measurement.

The experimental curves (expressed as mean of five replications) were described using the Gompertz mathematical model (the Curve Expert program, Version 1.37 for Windows) with the following formula:

$$y = a \cdot e^{-e^{b-x}}$$

a, b, c – equation coefficients of the model
 x – culturing time (h)
 y – changes in the wastewater electrical impedance (%).

Figure 1

On the basis of the Gompertz model data, high correlated with experimental data ($r > 0.95$), mathematical parameters indicating the dynamics of the electrical impedance changes were determined:

$$I_{max} - \text{maximal impedance changes rate}$$

$$I_{max} = \frac{(a \cdot c)}{e} \quad (\% \text{ of impedance changes } h^{-1}),$$

and

$$X_I - \text{time necessary to reach } I_{max}$$

$$X_I = \frac{b}{c} \quad (h).$$

The parameters were used for the comparative statistical analysis of the obtained impedimetric

curves (Figure 1). The proposed parameters constitute authors own modification of those elaborated by Zwietering (1993) and describe the dynamic of microbial growth (Lasik, 2004; Nowak *et al.*, 2005).

The process of wastewater biodegradation

The wastewater utilization process was performed in conditions of shaken cultures (130 rpm) for 72 h at 55°C. The biodegradation effectiveness was evaluated as reduction of Chemical Oxygen Demand (COD) after 72 h of the wastewater treatment. The determination of the COD involved the measurement of the quantity of oxygen derived from the potassium dichromate used for the oxidation of wastewater organic and inorganic compounds in the environment of sulphuric acid and in the presence of silver sulphate as a catalyser. It was carried out by using the spectral photometer CADAS 30s and LCK 014 cuvette tests (Dr Lange, Germany) (Anon., 2000).

Statistical analysis

Results were expressed as means ± SD for five replicates. The significance of differences between mean values was determined by one-way analysis of variance coupled with Duncan's test. The values described with $p < 0.05$ were considered as significant (Statistica 6.0). Correlation analysis was used to evaluate relationship between chemical (COD) and impedimetric (I_{max} and X_I) parameters describing the microbial ability for wastewater biodegradation.

Results

The analysis of the course of electrical impedance change curves, describing the dynamic of the biodegradation of wastewater components, showed a significantly higher ($p < 0.05$) metabolic activity of microorganisms occurring in the mixed cultures when compared to single isolates (Figure 2, 3). It was also found that some of the isolates, tested as single cultures, were characterized by a very weak

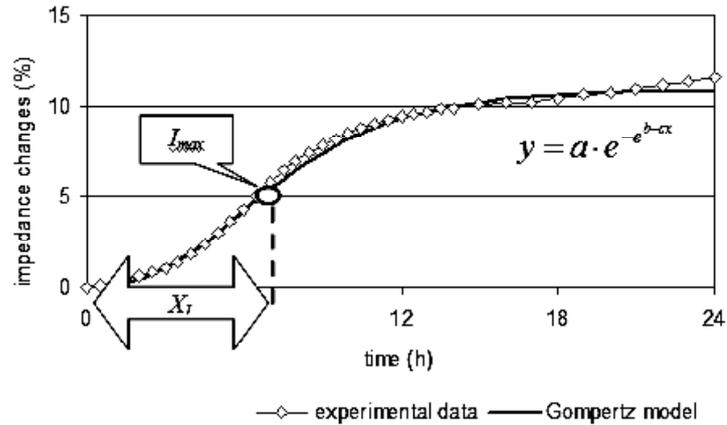


Figure 1. Mathematical description of the electrical impedance changes data obtained during bacterial wastewater biodegradation

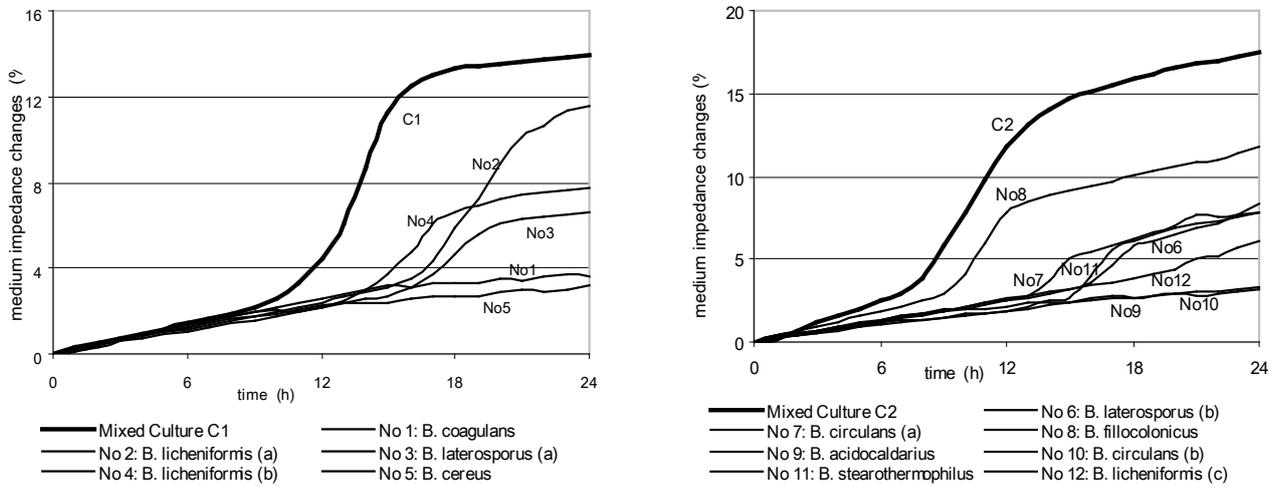


Figure 2. The course of electrical impedance changes during hot wastewater biodegradation by using of *Mixed Cultures C1* and *C2* and single isolates coming from them.

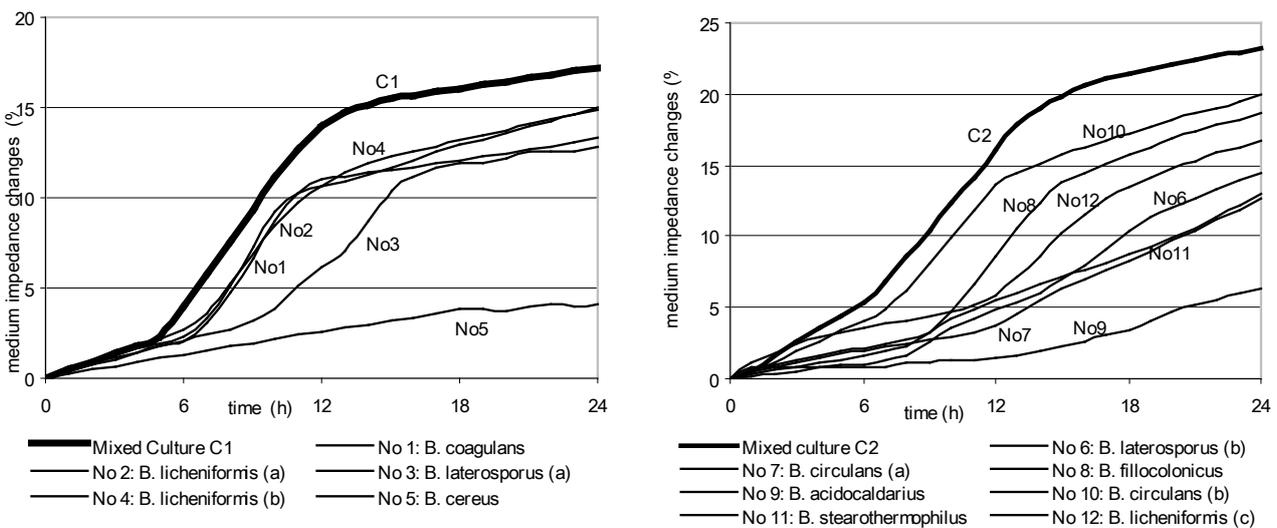


Figure 3. The course of electrical impedance changes during starch wastewater biodegradation by using of *Mixed Cultures C1* and *C2* and single isolates coming from them.

metabolic activity. Such low impedance signals were determined by the following isolates from *Mixed Culture C1*: *B. cereus* during biodegradation of both types of wastewater, and *B. coagulans* during biodegradation of hot wastewater, as well as those isolated from *Mixed Culture C2*: *B. acidocaldarius* during biodegradation of both types of wastewater, and *B. circulans (b)* during biodegradation of hot wastewater (Figure 2, 3).

The course of electrical impedance curves registered during bacteria wastewater biodegradation, was described mathematically by Gompertz microbial model (Figure 1). Afterwards, two impedimetric parameters: maximal impedance changes rate (I_{max}) and time necessary to reach I_{max} (X_l) were calculated for every tested microorganisms. Additionally, the ability for wastewater biodegradation of the tested bacteria was also evaluated as reduction of chemical oxygen demand (COD). The level of the wastewater COD reduction was next related to the impedimetric parameters of Gompertz mathematical model (I_{max} and X_l).

A linear correlation found between the level of the wastewater COD reduction and the values of I_{max} allowed to estimate the usefulness of this impedimetric parameter for a rapid technological determination of the microbial wastewater treatment capability. The linear correlation coefficient was $r = 0.89$ whereas, no correlation between COD reduction and the X_l parameter ($r = 0.59$) was found (Figure 4). Therefore, X_l parameter was excluded from the later experiments. However, I_{max} parameter was next applied for comparative analysis of the metabolic activity of the tested bacteria. On the basis of 95% confidence intervals of the means of both I_{max} and COD parameters, the tested isolates were divided into the following three groups: of high, middle and low metabolic activity (Table 2). The parameters characterized mixed cultures were not taken into consideration during statistical evaluation of single isolates activity. They were presented to illustrate significantly higher ($p < 0.05$) activity of mixed cultures in comparison with single isolates obtained from them.

According to the comparative analysis of isolates derived from both mixed cultures *C1* and *C2*, the microorganisms characterized with the high and middle values of I_{max} as well as with the high and middle capability for wastewater biodegradation, expressed as COD reduction, were selected. This resulted in the creation of two new bacteria mixed cultures, specific for each type of wastewater. According to this selection a new mixed culture specific for hot wastewater biodegradation was

composed. The new created population was named *Mixed Culture H* and included 8 strains: *B. licheniformis (a)*, *B. laterosporus (a)*, *B. licheniformis (b)*, *B. laterosporus (b)*, *B. circulans (a)*, *B. fillocolonicus*, *B. stearothermophilus* and *B. licheniformis (c)*. For the selected isolates the values of I_{max} ranged from 0.52 ± 0.06 to $1.07 \pm 0.04\%$ of impedance changes h^{-1} and the hot wastewater biodegradation reached up to 25% of COD reduction (Table 2). The second new mixed culture, created specific for starch wastewater biodegradation (named *Mixed Culture S*) included 10 strains: *B. coagulans*, *B. licheniformis (a)*, *B. laterosporus (a)*, *B. licheniformis (b)*, *B. laterosporus (b)*, *B. circulans (a)*, *B. fillocolonicus*, *B. circulans (b)*, *B. stearothermophilus*, and *B. licheniformis (c)*. For those isolates the values of the maximal impedance changes rate (I_{max}) ranged from 0.83 ± 0.03 to $1.28 \pm 0.02\%$ of impedance changes h^{-1} and the starch wastewater biodegradation achieved up to 33% of COD reduction (Table 2).

The microorganisms creating the new mixed cultures were acclimated to themselves for 2 weeks (5 passaging) during culturing on the adequate medium: hot wastewater for *Mixed Culture H* and starch wastewater for *Mixed Culture S*. The passages were performed every 3 days. The metabolic activity of new, specific mixed cultures: *Mixed Culture H* and *Mixed Culture S* were compared with the activity of *Mixed Culture C1* and *Mixed Culture C2*. It was found that both, the signals of impedance changes and the wastewater COD reduction, were significantly lower ($p < 0.05$) when the new *Mixed Cultures H* and *S* were applied. Lack of statistically significant differences ($p > 0.05$) was observed only when compared the biodegradation effect of hot wastewater using the *Mixed Culture H* ($26.3 \pm 3.5\%$ of COD reduction) and *Mixed Culture C1* ($28.4 \pm 2.5\%$ of COD reduction) (Table 2). The highest metabolic activity, expressed as dynamic of wastewater electrical impedance changes as well as the capability to reduce the wastewater load ($p < 0.05$), was shown by the *Mixed Culture C2* (Table 2).

The naturally developed qualitative and quantitative systems of microorganisms found in *Mixed Cultures C1* and *C2* were more active ($p < 0.05$) in comparison to the cultures developed in the laboratory. Despite the fact that in both, *C1* and *C2* *Mixed Culture*, there occurred isolates which – when tested individually – were characterized by a very low metabolic activity (Figure 2,3), an synergic effect of exactly such consortia of microorganisms present in natural conditions, was found to make the population significantly more effective in terms of wastewater

Table 2. The efficiency of the wastewater biodegradation obtained by using *Mixed Cultures* and single isolates, evaluated by values of impedimetric parameter (I_{max}) and COD reduction

Microorganisms	Hot wastewater		Starch wastewater	
	I_{max} (% of impedance changes h^{-1})	COD reduction (%)	I_{max} (% of impedance changes h^{-1})	COD reduction (%)
<i>Mixed Culture C1</i>	1.32 ± 0.06 ^B	28.4 ± 2.5 ^B	1.28 ± 0.05 ^B	33.8 ± 2.1 ^B
isolates:				
No 1 <i>B. coagulans</i>	0.24 ± 0.05 ^c	13.2 ± 1.4 ^b	1.13 ± 0.04 ^a	22.3 ± 2.1 ^b
No 2 <i>B. licheniformis</i> (a)	1.07 ± 0.04 ^a	19.3 ± 2.6 ^a	1.15 ± 0.02 ^a	27.5 ± 1.9 ^a
No 3 <i>B. laterosporus</i> (a)	0.63 ± 0.02 ^b	20.2 ± 3.1 ^a	0.98 ± 0.03 ^b	21.4 ± 2.0 ^b
No 4 <i>B. licheniformis</i> (b)	0.98 ± 0.03 ^a	19.7 ± 2.9 ^a	1.19 ± 0.03 ^a	24.1 ± 2.5 ^b
No 5 <i>B. cereus</i>	0.18 ± 0.02 ^c	11.3 ± 1.3 ^b	0.24 ± 0.01 ^c	17.7 ± 2.2 ^c
<i>Mixed Culture C2</i>	1.45 ± 0.03 ^A	34.2 ± 3.2 ^A	1.41 ± 0.01 ^A	36.1 ± 1.3 ^A
isolates:				
No 6 <i>B. laterosporus</i> (b)	0.81 ± 0.02 ^a	22.1 ± 3.2 ^a	0.91 ± 0.02 ^b	19.3 ± 1.1 ^c
No 7 <i>B. circulans</i> (a)	0.52 ± 0.06 ^b	19.6 ± 3.1 ^b	0.83 ± 0.03 ^b	20.6 ± 1.2 ^c
No 8 <i>B. filloclonicus</i>	0.94 ± 0.03 ^a	25.3 ± 3.0 ^a	1.11 ± 0.02 ^a	25.1 ± 1.3 ^b
No 9 <i>B. acidocaldarius</i>	0.18 ± 0.01 ^c	12.5 ± 3.2 ^c	0.41 ± 0.03 ^c	19.2 ± 1.4 ^c
No 10 <i>B. circulans</i> (b)	0.16 ± 0.01 ^c	14.4 ± 2.7 ^c	1.28 ± 0.02 ^a	33.1 ± 1.2 ^a
No 11 <i>B. stearothermophilus</i>	0.58 ± 0.03 ^b	20.6 ± 3.4 ^b	0.63 ± 0.01 ^c	26.3 ± 2.2 ^a
No 12 <i>B. licheniformis</i> (c)	0.23 ± 0.02 ^c	19.2 ± 2.6 ^b	0.92 ± 0.02 ^b	22.6 ± 1.7 ^b
New created mixed cultures				
<i>Mixed culture H</i>	1.15 ± 0.05 ^C	26.3 ± 3.5 ^B	not evaluated	not evaluated
<i>Mixed culture S</i>	not evaluated	not evaluated	1.09 ± 0.03 ^C	31.2 ± 2.2 ^C

Means and standard deviations for n = 5.

a, b, c - for single isolates evaluation: according to 95% confidence intervals of the mean values, the experimental data were divided into three groups expressed: high (a), middle (b) and low (c) metabolic activity, parameters of mixed cultures were not taken into consideration during statistical evaluation of single isolates activity.

A, B, C – for mixed cultures evaluation: according to Duncan's test unlike letters indicate significant differences ($p < 0.05$).

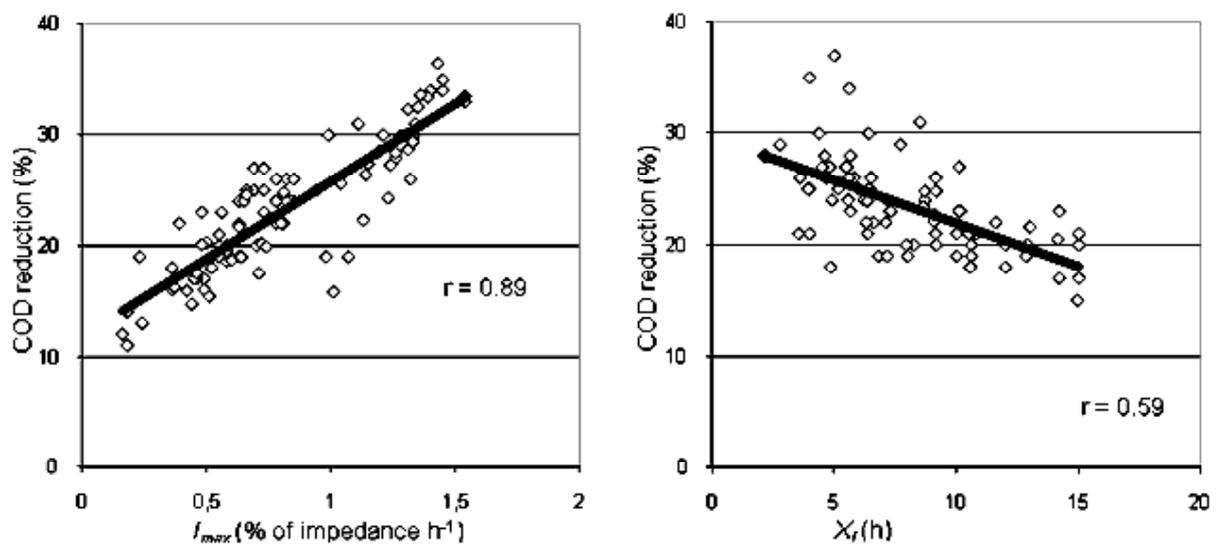


Figure 4. Correlations between the values of COD reduction obtained in the result of shaken cultures and parameters of the dynamics of electrical impedance changes (I_{max} and X_l) recorded during wastewater biodegradation by the tested microorganisms

bioremediation. The higher biodegradation potential of the tested cultures was always confirmed by both: COD reduction as well as by the impedimetric I_{max} parameter.

Discussion

The application of mixed cultures in processes of wastewater biodegradation has been described by a number of researchers. Most frequently, they were a mixed microbial populations isolated from surface waters and sewage (Sonnleitner and Fiechter, 1983; Lapara *et al.*, 2001; Lim *et al.*, 2001), composts (Blanc *et al.*, 1999; Fujio and Kume, 1991) as well as wastes from the food processing industry (Komukai-Nakamura *et al.*, 1996; Cibis *et al.*, 2002, 2006; Mohaibes and Heinonen-Tanski, 2004; Lasik and Nowak, 2007; Krzywonos *et al.*, 2008). Results of research on mixed cultures, concerning mainly mesophilic microorganisms, suggest that due to the synergistic interactions performed in microbial consortia, some species can initialing the biodegradation processes through the decomposition of a multiple components into smaller and simpler, which only in such form can be metabolized and assimilated by others microorganisms. Such symbiotic microbial relations were observed during polyvinyl alcohol degradation by using mixed microbial culture, in which the role of bacteria unable to degrade this compounds was to produce pyrroloquinoline quinone as an essential growth factor for the polyvinyl alcohol degrading bacterium (Sakazawa *et al.*, 1981; Shimao *et al.*, 1984). Similar symbiotic phenomenon were also described by using of mixed microbial population for biodegradation of organophosphorus insecticide (Gilbert *et al.*, 2003), terephthalate (Kimura and Ito, 2001), chloronitrobenzene (Park *et al.*, 1999), nitrate esters (Ramos *et al.*, 1996) and mixtures of volatile organic hydrocarbons (Komukai-Nakamura *et al.*, 1996; Oh and Bartha, 1997).

So far there is little know about synergistic relationships between thermophilic strains, especially during wastewater treatment. Investigations carried out by Surucu (1999) on the environmental requirements of thermophilic microorganisms participated in wastewater biodegradation processes showed that mixed cultures were characterized by considerably lower nutritional requirements in comparison with single microorganisms isolated from them. This phenomenon may also influence the higher activity of mixed cultures in the same environmental conditions in relation to single cultures of microorganisms. Taking it into consideration, construction a combined microorganisms consortium may be very useful in

specific functional microbial assemblages.

In the discussed investigations, the analysis of changes of the medium electrical impedance, indicating the dynamics of microbial metabolism, was employed for the rapid evaluation of the intensity of the wastewater components degradation. The discovery of the correlation between the impedimetric parameter I_{max} and the chemical evaluation of the efficiency of the biodegradation process (COD reduction) indicates the possibility to apply this method in experiments on the biological wastewater treatment. The results of the performed investigations confirmed the usefulness of the electrical impedance method for the rapid selection of microorganisms exhibiting high metabolic activity during their growth in specific environmental conditions. However, the development of new mixed cultures from the selected isolates, high active when cultured as single species, does not guarantee the creation of a mixed culture characterized by the metabolic activity higher than that developed in natural conditions, as it was shown in this experiment. Analyzing the impedance changes in wastewater during growth of single isolates there were observed a very low metabolic activity by some of tested species. However, the elimination of this microorganisms, when creating the new specific consortia, might caused reduction of the overall microbial activity. It is postulated, that their presence in mixed culture could perform a unique function in the microbial consortium and their lack could break the metabolic chain working completely only in the system of specific mixed culture.

The relatively low values of COD reduction (up to 36%), obtained in this study, were connected with high oxygen requirements of the applied microorganisms (Mohaibes and Heinonen-Tanski, 2004; Cibis *et al.*, 2006; Krzywonos *et al.*, 2008). In the conditions of shake flasks culturing the amount of dissolved oxygen was not sufficient. The application of the bioreactor with the possibilities of mixing and aeration of the media would be much useful. In this experiment, because of high number of the performed biodegradation processes, conducted parallel with impedimetric measurements, it was decided to use shake flask scale. The use of laboratory STR fermenter (stirred tank reactor) ensure the biodegradation effectiveness on the level of 70-80% of COD reduction. Additionally, to avoid the oxygen limitation during the aerobic processes, other operations like pure oxygen supplementation as well as wastewater dilution can be performed to significant increase the COD reduction (Nowak *et al.*, 2002; Rozih and Bordacs, 2002; Lasik, 2004; Cibis *et al.*, 2006; Lasik and Nowak, 2007).

The application of the impedance measurement method allowed eliminating meticulous, expensive and laborious microbiological, biochemical and genetic tests. The use of the proposed impedimetric parameter I_{max} permit easier and quicker, in comparison with classical COD measurements, evaluation of microbial biodegradation possibilities.

List of symbols:

y – changes in the electrical impedance of the wastewater

y_0 – value of the electrical impedance at the beginning of culturing

y_i – value of the electrical impedance at a given point of measurement.

a, b, c – equation coefficients of the model

x - culturing time (h)

I_{max} – maximal impedance changes rate (% of impedance changes h⁻¹),

X_I – time necessary to reach I_{max} (h)

Chemical Oxygen Demand (COD) [gO₂ l⁻¹]

Biochemical Oxygen Demand (BOD) [gO₂ l⁻¹]

Total Organic Carbon (TOC) [g l⁻¹]

Total nitrogen (TN) [g l⁻¹]

Total phosphate (TP) [g l⁻¹]

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