

Biodegradation of high temperature wastewater from potato starch industry

J. Nowak¹, M. Lasik¹, T. Miśkiewicz², Z. Czarnecki¹

¹*Institute of Food Technology, August Cieszkowski Agriculture University in Poznan, Poland.*

²*Department of Bioprocess Engineering, Oscar Lange Academy of Economics in Wroclaw, Poland.*

Abstract

The metabolic activity of mixed culture and single bacteria isolates got from it was investigated using Automatic Microorganisms Growth-Analyzer BacTrac 4100 and shake flask experiments using high temperature wastewater from potato industry. The single bacteria isolates metabolic activity and COD reduction of the wastewater was found always less than the mixed population they came from. The conclusion that the direction for optimising thermophilic biodegradation process is rather looking for more active mixed population than selection a single strain for the process led to investigations with other isolated mixed cultures. The process of thermophilic biodegradation is characterised by high oxygen demand so the shake flask experiments showed much less decrease of COD (up to 30%) than that made in the fermenter (up to 70%). The decrease of COD was especially intensive during the period of oxygen consumption by the bacteria for all the four mixed cultures used. Biodegradation of potato industry hot effluents by mixed cultures of thermophilic aerobic bacteria might be especially advantageous to pre-treatment high temperature waste streams without reducing their temperature.

1 Introduction

Poland faces the integration with European Community. The European Commission environmental law create the need for new technologies for the remediation of agro-industrial wastes and its stabilization and hygienization.

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 (Hasegawa et al. [1]; LaPara [2]).

Biodegradation with thermophilic aerobic bacteria may be useable in treating high-temperature wastewater from food industry, carrying large amounts of organic pollutants. Using high temperature wastewater allow to avoid the limiting factor preventing the widespread use of thermophilic biotechnology – the cost of raising reactor temperatures (Kosseva et al. [3]; Lim [4]; Skjelhaugen [5]).

Wastewater from enterprises manufacturing potato starch is characterised by very high biological oxygen demand (BOD₅) (of up to 26 g O₂/L) and COD (up to 77 g O₂/L) (Kutera et al. [6]). 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 (Malladi et al. [7]; Mason et al. [8]).

Attempts to apply thermophilic aerobic biological treatment of wastewater from potato processing industry were made by Malladi and Ingham [7], but the biological load of these wastes were relatively low. Wastes for breweries were investigated by Zvauya et al. [9]. Studies were also conducted to apply this method for the utilisation of molasses (Loll, [10]), wastewater from slaughterhouses (Couillard et al., [11]) and from dairy industry (Kosseva et al. [3]). 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 (Sonnleitner et al. [12]). Most commonly, the isolated organisms belonged to bacteria from genus *Bacillus* (Beaudet et al. [13]; Surucu et al. [14]).

The aim of the present work was to investigate the possibilities of biodegradation of high-temperature potato industry wastewater by thermophilic microflora.

2 Materials and methods

2.1 Materials

The material for investigations was wastewater after deproteinization of potato juices by the acid-thermal coagulation method (so called “hot” wastewater) from Wielkopolskie Przedsiębiorstwo Przemysłu Ziemniaczanego in Luboń, Poland. The material was collected during the 2000 autumn production campaign. The temperature of the collected wastewater ranged from 80-85°C, while its average chemical oxygen demand was 24.2 g O₂/L.

In additions, a special medium, for determination of the total number of microorganisms, employing the method of measurement of impedance changes (SY-LAB, Austria) called 001A, was also used.

2.2 Microorganisms

Investigations were carried out using the mixed thermophilic microbial cultures isolated in an industrial plant utilising the wastes from fruit-vegetable industry. The first mixed culture (P1) was isolated at 61°C and was used for BacTrac and shake culture experiments as well as single isolates obtained from it. Three other mixed cultures were used in laboratory fermenter experiments, one was isolated from the same plant but at 45°C (P2), the next one (L) was isolated from potato starch plant wastewater (WPPZ Luboń). Those three mixed cultures were adopted to growth on "hot" potato wastewater. The cultures were maintained through the regular passaging (every three days) onto a sterile wastewater substrate of pH = 7.0 (shake flask culture, temp. 55°C, 150 rpm). The 48 h culture prepared in this way was used for culture inoculation. The forth mixed culture (W) adopted originally for distillery stillage biodegradation was obtain from Oscar Lange Academy of Economics in Wroclaw, Poland.

Single isolates were stored on agar slants (Plate Count Agar – Merck) at 4°C.

A majority of single species in those four mixed cultures were bacteria of *Bacillus* genus (paper in preparation).

2.3 Methods

2.3.1 Chemical composition of "hot" potato wastewater

Chemical composition of the "hot" potato wastewater were done using following methods : pH (by potentiometric method), TOC, COD, (by Dr Lange cuvette test), total nitrogen (Kjeldahl method), protein nitrogen (precipitation by 10% trichloroacetic acid and than by Kjeldahl method), reducing substances as glucose (3,5-DNS colorimetric method), total reducing substances (hydrolisation by HCl and 3,5- DNS colorimetric method), dry matter (drying at 105°C), ash (mineralisation at 600°C), microelements and metals according to Polish Official Methods (PN).

2.3.2 Estimation of metabolic activity using the BacTrac 4100 Automatic Microorganisms Growth Analyzer

The assessment of the metabolic activity of tested microorganisms was performed employing the method of measurement of electrical impedance changes using the BacTrac 4100 Automatic Analyzer of Microorganism Growth (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 electric current and consequently, reduce environmental impedance. 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 curve of growth of microorganisms (Futschik et al. [15]).

The measurement of impedance changes was carried out at 55°C in measuring cells of the BacTrac 4100. 9 ml of culture medium (001A or wastewater) was placed in each cell and inoculated with 1 ml inoculum. All results are means from five replicates.

2.3.3 Wastewater biodegradation in conditions of shake flask culture

Cultures were conducted in conditions of shaken cultures (150 rpm) for 72 hours at 55°C on sterile wastewater whose initial pH 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 (Miller, [16]) and changes in COD index – by colorimetric method using the Dr Lange cuvette test.

2.3.4 Wastewater biodegradation in 2L laboratory fermenter

Batch aerated fermentations using mixed population P1, P2, L and W were run using 1.5 L working volume Biostat B bioreactor (B.Braun). Aeration was at 1.5 vvm and mixing was achieved using a stirrer speed of 550 rev/min. pH was kept constant by addition of 2.5 M H₂SO₄ and 2.5 M NaOH. The optimal value of pH was investigated earlier (Nowak et al. [17]). For the L mixed culture, the potato wastewater was fortified with 20 g/L glucose for the culture growth promoting.

3 Result and discussion

The chemical composition of the hot wastes from protein recovery fluctuated during producing campaign. The results of the analyses from 2000 year fall campaign are presented in Tab 1. As shown by these data, the basic chemical parameters of the hot wastewater were relatively stable (d.m., COD, pH, reducing substances, total and protein nitrogen), while the levels of macro- and microelements varied considerably. This may be attributed primarily to changes of raw material during producing campaign.

The metabolic activity of P1 mixed culture of microorganisms as well as of single isolates derived from it was assessed during culturing on 001A microbiological medium and on wastewater (Fig. 1). 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 (Tyszkla et al. [18]).

Tab. 1 Chemical composition of “hot” potato wastewater after protein recovery

Parameter	Range
COD (mg O ₂ /L)	20563 – 27608
pH	4.6 – 5.88
Dry matter (g/L)	29.02 – 37.22
Reducing substances (g/L)	2.15 – 4.99
Total sugars (g/L)	4.65 – 8.66
Total nitrogen (g/L)	1.49 – 2.11
Protein nitrogen (g/L)	0.16 – 0.47
Ash (g/L)	6.57 - 10.79
Chlorides (mg Cl/L)	1350 - 2550
Sulphates (mg/L)	520 - 680
Total Phosphates (mg PO ₄ /L)	164 - 538
Potassium (mg K/L)	180 - 3860
Calcium (mg Ca/L)	154 - 400
Sodium (mg Na/L)	84 - 210
Magnesium (mg Mg/L)	21.7 - 250
Zinc (mg Zn/L)	2.21 - 2.40
Manganese (mg Mn/L)	1.11 - 2.35
Copper (mg Cu/L)	0.518 - 0.589
Total Iron (mg Fe/L)	0.1 - 3.76
Nickel (mg Ni/L)	0.1 - 0.145
Lead (mg Pb/L)	0.079 - 1.38
Chromium (mg Cr/L)	0.075 - 0.183
Cadmium (mg Cd/L)	0.016 - 0.054
Mercury (mg Hg/L)	0.0004

The preliminary studies of the wastewater biodegradation were carried out in shake flasks. The duration of the process was 3 days. pH of the wastewater was brought to 7.0 on the start but during the fermentation pH was not regulated.

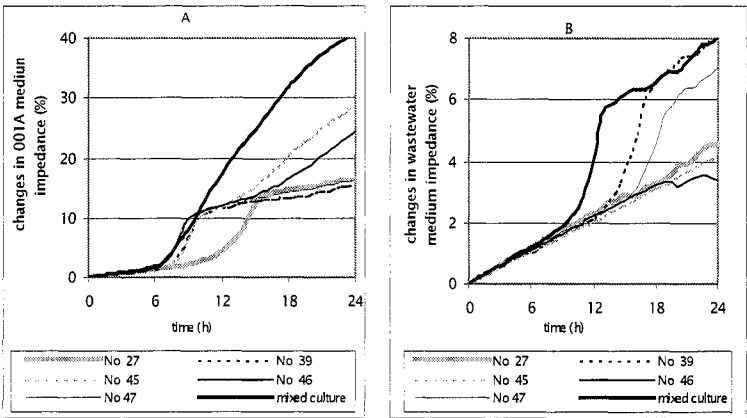


Fig. 1: Changes in electrical impedance of 001A medium (A) and “hot” potato wastewater (B) during growth of single isolates and P1 mixed bacteria population

The pH changes are shown on Fig. 2. In all tests, both for mixed culture and single isolates, pH increased to the value of 9-9,5. The biodegradation of the waste (COD reduction) was distinctly better for mixed culture than for single isolates, but the decrease did not exceed 30% (Fig. 3). Similarly, the utilisation of reducing substances from wastewater was better when mixed culture was applied (Fig. 4).

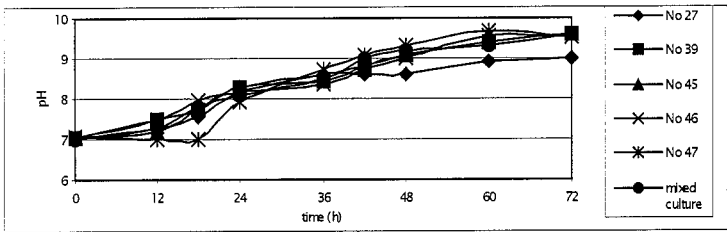


Fig. 2: Changes of pH of “hot” potato wastewater during fermentation by single isolates and P1 mixed culture

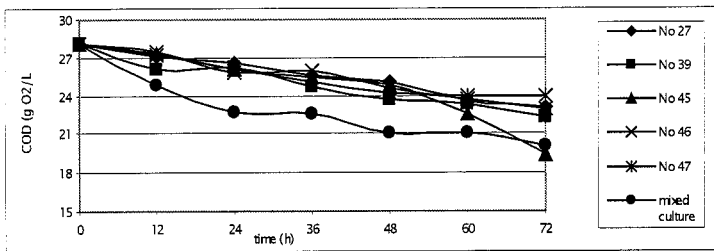


Fig. 3: Potato wastewater COD changes during fermentation by single isolates and P1 mixed culture

The availability of oxygen is much better in laboratory stirred-tank fermenters, owing to the higher power input possible through agitation. The control of other parameters essential for thermophilic bacteria growth is also much more precise.

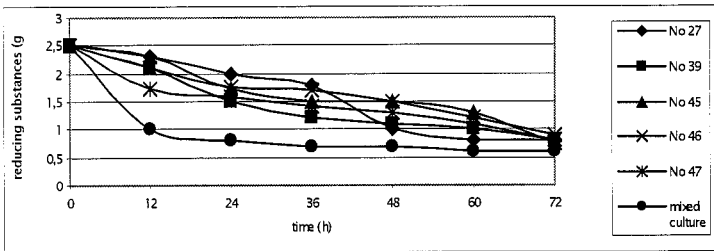


Fig. 4: Changes in the content of reducing substances in “hot” potato wastewater during fermentation by single isolates and P1 mixed culture

For this reasons, biodegradation processes using four mixed cultures were carried out in the 2L bioreactor with wastewater as a medium. The biodegradation process took a different course for the all used mixed cultures.

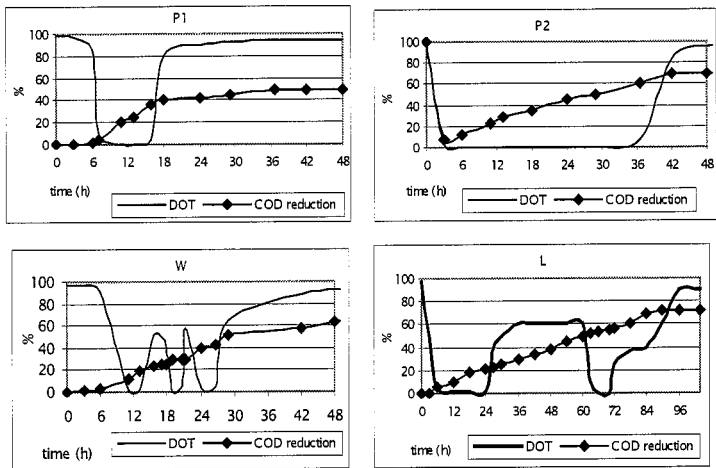


Fig. 5: The course of dissolved oxygen concentration (DOT) and COD reduction during potato wastewater degradation by four mixed cultures (P1, P2, W, L)

The analysis of the dissolved oxygen tension (DOT) profile, revealed that the decrease of COD (expressed as % of COD reduction) was especially intensive during the period of oxygen consumption by the bacteria for all the four mixed cultures (Fig 5).

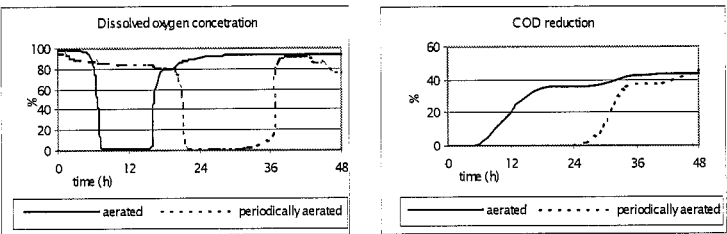


Fig. 6: Changes of DOT (%) and COD reduction (%) during constantly and periodically aerated fermentation of P1 mixed culture at 55°C

The process was faster and more efficient at 45°C and the reduction of COD and other components varied considerably higher at 45°C (Tab. 2). The waste is foaming profoundly during Biostat fermentation, especially on the beginning of the process. To avoid the use of large quantity of antifoam substances (it increases COD) we tried to start the process without aeration and started to add the air in the time when oxygen present in the medium was consumed. The final

COD reduction for constantly aerated and periodically aerated processes were similar, but the speed of COD reduction was much different (Fig. 6)

Tab.2: Changes in potato wastewater composition during biodegradation in laboratory fermenter by mixed cultures

Parameter		Type of fermentation			
		P1 (55°C)	P2 (45°C)	W (45°C)	L (45°C)
COD (mg/L)	initial	29 159	33 984	28 432	51 459
	final	16 270	11 206	9 425	14 436
	% reduction	44.2	67.1	66.8	71.9
Reducing Substances (g/L)	initial	2.98	2.86	2.73	20.04
	final	0.69	0.71	1.03	1.37
	% reduction	76.8	75.3	62.3	93.2
Total Nitrogen (mg/L)	initial	2 423	2 106	2 474	1 794
	final	2 154	1 700	2 120	1 653
	% reduction	11.1	19.3	14.3	7.8
Total Phosphates (mg/L)	initial	466	412	471	538
	final	431	377	446	397
	% reduction	7.5	8.5	5.3	26.2

4 Conclusion

The single bacteria isolates (mainly *Bacillus* strains) metabolic activity assayed using Bac-Trac analyzer was found always less than the mixed population they came from. The results were similar in shake flask cultures using “hot” potato wastewater.

The process is characterised by high oxygen demand and the shake flask experiments showed much less decrease of COD than that made in the fermenter. Thermophilic microorganisms mixed cultures isolated from fruit-vegetable wastes and from starch potato processing were used to carry out the biodegradation process in 2 L laboratory fermenter. The COD levels of “hot” wastewater decreased up to 70% during thermophilic remediation. Biodegradation of potato industry hot effluents by mixed cultures of thermophilic aerobic bacteria can be regarded as an effective method. It might be especially advantageous to short-time pre-cleaning of high temperature waste streams without reducing their temperature.

Acknowledgements

The authors wish to thank the European Commission for the financial support of their studies under Project QLK3-1999-00004 of the FRAMEWORK V Program.

References

- [1] 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), 163-169, 2000.
- [2] LaPara T.M., Alleman J.E. Thermophilic aerobic biological wastewater treatment. *Wat. Res.* 33 (4), 895-908, 1999.

- [3] 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, 1675-1679, 2001.
- [4] 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), 131-137, 2001.
- [5] Skjelhaugen O.J. A Farmer-operated system for recycling organic wastes. *J. Agric. Engng. Res.* 73, 373-382, 1999.
- [6] 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.
- [7] Malladi B., Ingham S.C. Thermophilic aerobic treatment of potato-processing wastewater. *W. Jour. of Microbiol. And Biotechnol.* 9, 45-49, 1993.
- [8] Mason C.A., Haner A., Hamer G. Aerobic thermophilic waste sludge treatment. *Wat. Sci. Tech.* 25 (1), 113-118, 1992.
- [9] Zvauya R., Parawira W., Mawadza C., Aspects of aerobic thermophilic treatment of Zimbabwean traditional opaque-beer brewery wastewater. *Bioresource Technol.*, 48, 273-274, 1994.
- [10] Loll U. Purification of concentrated organic wastewaters from the foodstuffs industry by means of aerobic-thermophilic degradation process. *Progr. Wat. Technol.* 48 (2/3), 373-379, 1976.
- [11] Couillard D., Zhu S. Thermophilic aerobic process for the treatment of slaughterhouse effluents with protein recovery. *Envir. Pollut.* 79, 121-126, 1993.
- [12] Sonnleitner B., Fiechter A. Bacterial diversity in thermophilic aerobic sewage sludge. Active biomass and its fluctuations. *Eur. J. Appl. Microbiol. Biotechnol.* 18, 47-51, 1983.
- [13] Beaudet R., Gagnon C., Bisaillon J.G., Ishaque M. Microbial aspect of aerobic thermophilic treatment of swine waste. *Appl. Environ. Microbiol.* 56 (4), 971-976, 1990.
- [14] 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, 1639-1662, 1975.
- [15] Futschik K., Pfutzner H., Doblander A., Asperger H. Automatic registration of microorganism growth by new impedance method. *Abstr. Intern. Meetg. Chem. Eng & Biotechnol. Achema*, 88-91, 1, 1988.
- [16] Miller G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar; *Analytical chemistry*, 31 (3) 426-428, 1959.
- [17] Nowak J., Lasik M., Czarnecki Z., Wykrywanie obecności oraz charakterystyka aktywności metabolicznej bakterii poprzez badanie zmian impedancji elektrycznej. *Aparatura Badawcza i Dydaktyczna*, 1, 2002
- [18] Tyszkla M., Kaszycki P., Kołoczek H., Metody współczesnej biotechnologii w procesach biodegradacji zanieczyszczeń. *Ekologia i Technika*. 6 (6), 175-179, 1998.