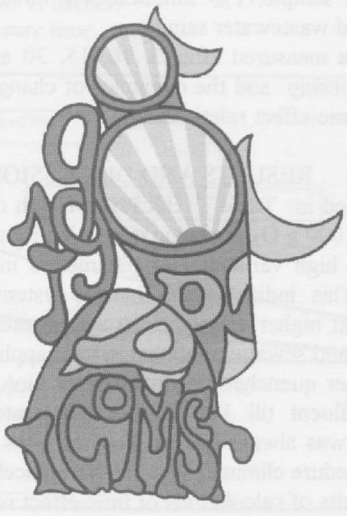
PS 14

Poster session and workshop 14

Animal welfare and environmental issues



Thursday, September 3rd
17:15h-18:45h

AN APPROACH TO THE USE OF BIOLUMINIESCENCE TECHNIQUE FOR DETERMINATION OF BIOTOXICITY OF WASTEWATER FROM MEAT PROCESSING PLANT

PIOTR KONIECZNY, WALDEMAR UCHMAN, JOANNA MATKOWSKA,

Institute of Meat Technology of Poznań , Agricultural University, Poland

BACKGROUND

Organic substances, pathogen organisms and toxins present in wastewater from meat processing plant demonstrate high potential risk for the environment. Therefore monitoring of toxicity of wastewater becomes regular and in some countries obligatory practice (1). There is a number of methods of determination of bio-toxicity of wastewater, however the bioluminescent technique seems to be promising. The method is based on quenching the bioluminescence of testing bacteria by the toxins present in wastewater. The quenching results from the interference of hazardous chemical with biochemical and enzymatic system of bacteria, thus the regular metabolism is damaged. In consequence, intensity of bioluminescence decreases (4).

Bioluminescence technique has been practically applied for rapid monitoring of hygiene of food-contact surfaces (2) and the determination of microbial contamination of food products. Also, the use of this technique for determination of efficiency of biocides has been reported (3). With regard to toxic substances present in water, ISO/CD 11348 recommends the use *Vibrio fischeri* as a testing bacteria (5). In this paper preliminary results of bioluminescent determination of toxicity of wastewater from meat processing plants are presented.

OBJECTIVES

The experiment was undertaken to determine the influence of wastewater from meat processing plant on bioluminescence of *Vibrio fischeri*. The bioluminescent data were compared with major parameters of raw wastewater samples and pre-treated ones at meat plant site.

MATERIALS AND METHODS

Both raw and pre-treated samples of wastewater were collected from meat processing plant of slaughter capacity of 800 hogs/day. Preliminary treatment involved the use of dissolved air flotation system (DAF) and coagulation with $Fe_2(SO_4)_3$. Only after treatment the effluent can be discharged into sewage municipal system. Wastewater samples from municipal treatment plant were also taken for the comparison.

The parameters of wastewater samples were determined in accordance to Polish Standards and these were: pH, COD (Chemical Oxygen Demand), conductivity, the chloride, sulphate, ammonia nitrogen content and total amount of dissolved substances.

The suspension of *Vibrio fischeri* (BioTox ™ System) was mixed with equal volume of wastewater samples and left for 30 min. long exposure. Then the intensity of luminescence was measured using the luminometer type 1253 (BioOrbit, Finland). To avoid turbidity, the wastewater samples were filtered through fine glass wool before mixing with the bacteria suspension. The inhibition coefficient was calculated using the formula:

$$INH\ (\%) = 100 - (IT_{30} / KF*IT_o) * 100$$

were:

KF - correction factor = IC_{30} / IC_o , IC_{30} - luminescence intensity after 30 min exposure, in relative luminescence units (RLU),

IC_o - initial luminescence intensity of the control sample, IT_{30}- luminescence intensity of tested wastewater sample after 30 min exposure IT_o - initial luminescence intensity of tested wastewater sample.

In addition, the bioluminescence of samples were measured after 5, 10, 15, 30 and 60 min. of exposure of testing bacteria to wastewater samples to determine time-effect relationship and the dynamics of changes of bioluminescence. An exponential model of the type of $y=A*\exp(b*t)$ was applied to describe time-effect relationship.

RESULTS AND DISCUSSION

The parameters of wastewater samples are presented in Table 1. Because of high content of organic substances (fat, proteins), the COD values of raw wastewater were high - above 1000 g O_2 per cubic meter. Various and unpredictable technological situations in the meat processing plant were reflected in relatively high variability of parameters measured, nevertheless the parameters of treated effluents correspond well with literature data (1). This indicates also that the system for wastewater treatment in the meat plant was operating effectively enough. This is interesting that higher ammonia nitrogen content was found in municipal effluent - presumably due to differences in raw wastewater characteristic and sewage treatment system applied.

It was found, that all samples of wastewater quenched the intensity of bioluminescence emitted by *Vibrio fischeri* and the inhibition varied from 64.9 % for municipal effluent till 10.9 % for pre-treated wastewater from meat plant. Inhibition of bioluminescence by raw effluent from meat plant was always higher than that for pre-treated effluent (e.g. 61.8 % and 38.0 % , respectively). This suggests that the treatment procedure eliminates certain substances interfering the emission of luminescence by test bacteria. These data correspond very well with results of calculations of time-effect relationship (Figure1). This can be computed from the formulas, that time required for quenching the bioluminescence intensity to 50% of initial value was 23.10 min for pre-treated samples and 15.06 min for raw, not treated wastewater samples.

The results obtained do not permit the calculations of more sophisticated relationships, e.g. the correlation between the quenching effect and the parameters of wastewater samples. In general , the bioluminescence emitted by *Vibrio fischeri* should be considered as the effect influenced by many factors. Probably other substances present in wastewater and not controlled in this experiment interfered the bioluminescence reaction .It is also possible that these substances could also stimulate this process. For this reason the further studies would be needed.

CONCLUSIONS

This arises from preliminary experiment that bioluminescent test with *Vibrio fischeri* can be applied for determination of bio-toxicity of wastewater effluents from meat processing plant. Using this test significant differences between raw and pre-treated wastewater were found. The reduction of bioluminescence intensity till 50% of initial value occurred after 15 min of exposure of testing bacteria to raw, not treated wastewater, in comparison with 23 min for pre-treated effluents. Nevertheless, practical utilisation of this technique would depend on performance of tests under the conditions prevailing in particular meat processing plant.

REFERENCES

1) Brechtelsbauer P., (1994) , „Abwasserbehandlung und Feststoffbehandlung im Schlachthof" , Fleisch, 48, 5 , 398
2) Kircher D et al.(1996): "Eignung eines Biolumineszenzverfahrens zur Uberprufung der Reinigung und Desinfektion im Bereich der Lebensmittelverarbeitung", Fleischwirtschaft, 76 (9), 897-903,
3) Mansel W. Griffiths,(1995) „Bioluminescence And The Food Industry".J. Rapid Methods and Automation in Microb.,4, 65-75.
4) „Handbook of Bioluminescent Toxicity Screening(1996): A Practical Guide to the Effective Use of the Bio-Orbit BIOTOX™ System" (unpublished)
5) ISO/CD (draft) 11348,1994-09, : „Determination of the Inhibitory Effect of Water Samples on the Light of *Vibrio fischeri*"

Table 1. Characteristics of wastewater samples and inhibition coefficient calculated from biolouminescent test.

Parameter	Raw meat plant wastewater	Pre-treated meat plant wastewater	Treated municipal wastewater
pH- value	7.00 - 7.10	6.80 - 7.10	7.30 -7.40
COD (mg O_2/dm^3)	1075 – 1190	260 - 680	315 – 339
Sulphate content (mg/dm^3)	140 – 150	260 - 400	100 – 150
Ammonia nitrogen (mg/dm^3)	4.76 - 5.60	1.12 - 4.76	28.36 – 30.90
Chloride content (mg/dm^3)	170 – 190	180 - 250	100 – 126
Total dissolved substances (mg/dm^3)	700 – 846	1060 - 1396	813 – 839
Conductivity (μS)	1403 – 1435	1300 - 1962	1300 – 1320
Inhibition coefficient (INH%)	44.3 – 61.8	10.9 – 38.8	61.3 – 64.9

Fig.1.Bioluminescence of V. fischeri as function of exposure time.