ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Technol. Aliment. 8(4) 2009, 33-38*
ISSN 1644-0730 (print) ISSN 1889-9594 (online)

AMMONIA DISINFECTION OF CORN GRAINS INTENDED FOR ETHANOL FERMENTATION*

Magdalena Broda, Włodzimierz Grajek
Poznań University of Life Sciences

Background. Bacterial contamination is an ongoing problem for commercial bioethanol plants. It concerns factories using grain and also other raw materials for ethanol fermentation. Bacteria compete with precious yeasts for sugar substrates and micronutrients, secrete lactic and acetic acids, which are toxic for yeast and this competition leads to significant decrease of bioethanol productivity. For this study, bacterial contamination of corn grain was examined. Then the grain was treated by ammonia solution to reduce microbial pollution and after that the microbiological purity of grain was tested one more time. Disinfected and non-disinfected corn grains were ground and fermentation process was performed. Microbiological purity of this process and ethanol yield was checked out.
Material and methods. The grain was disinfected by ammonia solution for two weeks. Then the grain was milled and used as a raw material for the ethanol fermentation. The fermentation process was carried out in 500-ml Erlenmeyer flasks. Samples were withdrawn for analysis at 0, 24, 48, 72 hrs. The number of total viable bacteria, lactic acid bacteria, acetic acid bacteria, anaerobic bacteria and the quantity of yeasts and moulds were signified by plate method.
Results. Ammonia solution effectively reduces bacterial contamination of corn grain. Mash from grain disinfected by ammonia contains less undesirable microorganisms than mash from crude grain. Moreover, ethanol yield from disinfected grain is at the highest level.
Conclusions. The ammonia solution proved to be a good disinfection agent for grain used as a raw material for bioethanol fermentation process.

Key words: ammonia, grain, disinfection, bioethanol fermentation

*This work was supported by the Polish Ministry of Science and Higher Education as a R&D project from the founds supported research in 2007-2010 (grant R&D No. 0619/P01/2007/02) and as a Polish POST-DOC III project (PBZ/MNiSW/07/2006/18).

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Corresponding author – Adres do korespondencji: Dr Magdalena Broda, Department of Biotechnology and Food Microbiology of Poznań University of Life Sciences, Wojska Polskiego 48, 60-627 Poznań, Poland, e-mail: mbroda@up.poznan.pl

INTRODUCTION

There is currently a high level of interest in a renewable energy resources, especially ethanol as biofuel. Global crude oil reserve is a finite source, and its depletion is occurring much faster than previously predicted, so bioethanol is believed to be one of the best alternatives. In many developed countries the use of bioethanol as an alternative fuel in the amounts up to 15% is highly recommended. In UE a new directive 2003/30/EC was accepted in November 2001, that requires of member states to establish legislation about utilization fuels from renewable resources. Utilization should cover 2% of the total fuel consumption in 2005 and 5.75% in 2010 [Bomb 2007].

Ethanol is a good transportation fuel. It contains 35% oxygen, so it is an oxygenated fuel, which reduces NOx and particulate emission from combustion. It can be used as a blend with gasoline (10% is used in the US and 22% is used in Brazil) or individually (95% ethanol and 5% water) in specially designed engines. Using bioethanol as a fuel provides a lot of environmental benefits, such as reduction of air pollution and accumulation of carbon dioxide in the atmosphere or reduction of carbon monoxide and ozone formation [Prasad et al. 2007].

The raw materials to ethanol fermentation could be not only crop and edible plants, but also many kinds of lignocellulosic materials: crop residues, grasses, sawdust, wood chips, solid animal waste, industrial and community wastes. Using such materials is economically and environmentally profitable. Additionally, the modern technologies are invented, based on new enzymes, which are able to hydrolyze starch without former mashing and fluidization in high temperatures. All this exposes to risk of high microbial contamination, and it leads to significant limitation of ethanol production. Therefore, recognition and reduction of microbial contamination, as well as prevention of such infections, are essential and need deeper understanding [Rosillo-Calle and Walter 2006].

Ammonia is known as a disinfection agent for fungal and bacterial contamination, it also reduces mycotoxins produced by moulds. Moreover, it is a very attractive substance, because of its low purchase cost. It can be a source of nitrogen for yeast in the fermentation process, increasing the level of valuable proteins in fermentation's leavings [Tajkarimi et al. 2008].

The present study examines the possibility of ammonia acid solution to be used for disinfection of corn grain intended for modern technology of bioethanol fermentation.

MATERIAL AND METHODS

Corn grain and flour

Corn grain was obtained from agricultural distillery in Swadzim (the Wielkopolska Region, Poland). Flour used in fermentation process was made by milling the corn grain in Retsch SM100 grinder.

Disinfection process

The grain was disinfected by ammonia solution (total ammonia concentrations in tested probes were 0.5%, 1% i 1.5% w/v). This process was performed in sterile

closed jars, samples for microbial analysis were collected at start, 1 day, 1 week and two weeks of disinfection process. The grain was then ventilated in sterile conditions, milled and used as a raw material for the ethanol fermentation.

Fermentation conditions

The fermentation process was carried out in 500-ml Erlenmeyer flasks containing 200 ml of corn mash, during three days, without recirculation. Before the fermentation mash was adjusted to 3.5 pH using concentrated H_2SO_4. The fermentation was processed with a new technology without mashing at high temperature. Only amylolytic enzymes were used to starch decompose (STARGEN 001 with an activity of ≥ 456 GSHU/g «GSHU = granular starch hydrolyzing units»). Samples were withdrawn for analysis at 0, 24, 48, 72 hrs. Ethanol concentration and the number of total viable bacteria, lactic acid bacteria, acetic acid bacteria, anaerobic bacteria and the quantity of yeasts and moulds were signified.

Estimation of the level of microbiological infection of raw materials and mashes

The grain was immersed in physiological salt and shaken out for 3 hrs. The total number of microorganisms (cultured medium: NUTRIENT LAB-AGAR supplemented with 2% (w/v) glucose), the number of lactic acid bacteria (cultured medium: MRS LAB-AGAR), acetic acid bacteria (cultured medium: MALT EXTRACT LAB-AGAR supplemented with 2% (v/v) ethanol and a drop of glacial acetic acid), the number of anaerobic bacteria (cultured medium: THIOGLYCOLATE FLUID MEDIUM with 2% agar) and the quantity of yeasts and moulds (cultured medium: CHLORAMPHENICOL LAB-AGAR) in raw materials and mashes were quantified by counting the colony-forming units (CFU) after 48 hrs. In case of mash, cykloheximide (SIGMA) 100 mg/ml was added to medium to kill yeasts and make it possible to count alive bacterial cells. All microbiological media were from BIOCORP company.

RESULTS AND DISCUSSION

Grain disinfection by ammonia solutions

The ammonia occurred as a good disinfection agent for microbial contamination of corn grain (results shown in Table 1). The best effects were obtained for disinfection by 1.5% ammonia solution. This solution effectively removed moulds and yeasts and remarkably decreased the amount of all tested bacteria groups. 0.5% ammonia solution was the least effective, as expected. Good results were received for 1% ammonia solution: it effectively removed moulds and yeasts already after one week of incubation and lactic acid bacteria after two weeks of incubation. It also remarkably increased the amount of total viable bacteria, acetic acid bacteria and anaerobic bacteria. This solution seems to be good enough for further experiments (the fermentation) because of its sufficient disinfection action. What is more, the concentration of 1% ammonia should not interrupt the fermentation process. Obtained results are compatible with effects got by other scientists, who investigated the influence of ammonia solution on microbial contamination of animal feeds [Tajkarimi et al. 2008, Khan et al. 1995].

Table. 1. Grains disinfection by ammonia solutions. The number of bacteria is in log CFU/g

Groups of bacteria	Time of samples withdrawn	Pure grains	Grain treated by 0.5% ammonia	Grain treated by 1.0% ammonia	Grain treated by 1.5% ammonia
Total viable bacteria	s	5.95	5.95	5.95	5.95
	1d	6.31	5.91	5.31	4.85
	1w	5.95	4.56	4.81	4.65
	2w	5.26	4.56	4.56	4.26
Lactic acid bacteria	s	4.56	4.56	4.56	4.56
	1d	7.21	4.26	4.26	3.26
	1w	5.80	4.56	4.35	3.95
	2w	4.95	2.90	0	0
Acetic acid bacteria	s	4.43	4.43	4.43	4.43
	1d	4.57	4.48	3.91	4.12
	1w	4.77	3.95	3.56	3.32
	2w	6.25	2.47	2.91	2.87
Moulds and yeasts	s	4.95	4.95	4.95	4.95
	1d	6.56	2.73	1.95	0
	1w	5.86	0	0	0
	2w	5.25	0	0	0
Anaerobic bacteria	s	4.25	4.25	4.25	4.25
	1d	4.60	4.48	4.48	4.24
	1w	4.83	5.91	4.95	4.73
	2w	5.13	4.47	4.25	3.90

The fermentation

Milled disinfected and non-disinfected grains were used for ethanol fermentation process. The aim of this experiment was to compare the ethanol productivity and the level of microbial contamination in disinfected and non-disinfected grains fermentation. The results are shown in Figure 1. The rate of microbial contamination of fermentation from non-disinfected grains is higher than from ammonia disinfected ones, as assumed. It concerns all tested bacteria groups with the exception of acetic acid bacteria, which are at the comparable level. The primary bacterial contaminants of fermentation are lactic acid bacteria. Contamination by this group of microorganisms is a common occurrence during the fermentation process [Schell et al. 2007, Skinner and Leathers 2004]. Moreover, it is also the most dangerous because the fermentation conditions are optimal for their growth and increase of their number leads to decrease of ethanol production and makes economical losses.

The ethanol productivity from disinfected grain is higher than from non-disinfected one. The difference is not very significant (about 2% v/v), but the experiment was performed on the laboratory scale and in a large scale fermentation it would be economically important.

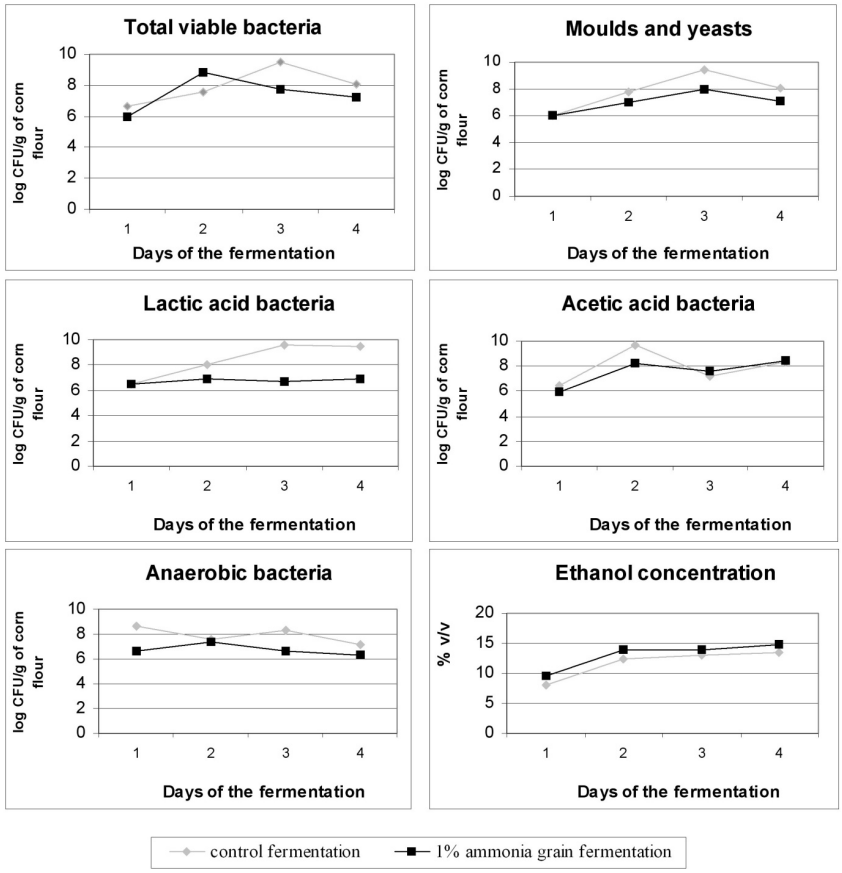

Fig. 1. Microbial infections of the fermentation process

CONCLUSIONS

The results of our study show that ammonia solution is a good disinfection agent for corn grains meant to bioethanol fermentation. Anyhow, it does not completely eliminate bacterial contamination, but it limits the process to a high degree. Grains decontamination results in an increase of ethanol productivity. Bacterial infections in fermentation process, although expected and tolerated, are not desired because of economical considerations. It is very important to find the way to completely eliminate this pollution to prevent stuck of fermentation process and make it more profitable.

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DEZYNFEKCJA ZA POMOCĄ AMONIAKU ZIARNA ZBÓŻ PRZEZNACZONEGO DO FERMENTACJI

Wstęp. Zanieczyszczenia bakteryjne są poważnym problemem w przemysłowych gorzelniach produkujących bioetanol. Trudności dotyczą gorzelni wykorzystujących jako surowiec do fermentacji nie tylko ziarno zbóż, ale również inne surowce. Niepożądane bakterie współzawodniczą z drożdżami szlachetnymi o substraty odżywcze i cukry, wydzielają kwasy octowy i mlekowy, które są toksyczne dla drożdży. Współzawodnictwo to prowadzi do znaczącego zmniejszenia produktywności bioetanolu. Celem pracy było zbadanie mikroflory zanieczyszczającej ziarno kukurydzy. Ziarno traktowano roztworem amoniaku w celu redukcji niepożądanych mikroorganizmów, po czym ponownie analizowano poziom zanieczyszczenia. Odkażone i nieodkażone ziarno kukurydzy zostało zmielone i poddane procesowi fermentacji. Określono czystość mikrobiologiczną procesu oraz wydajność etanolu.
Materiał i metody. Ziarno odkażano roztworem amoniaku przez dwa tygodnie, następnie zmielono i użyto jako surowiec w procesie fermentacji etanolowej. Fermentacje prowadzono w kolbach typu Erlenmeyer o pojemności 500 ml. Próbki do analizy pobierano po 0, 24, 48, 72 h fermentacji. Ogólną liczbę bakterii, liczebność bakterii mlekowych, octowych oraz beztlenowych, a także grzybów i pleśni oznaczono metodą płytkową.
Wyniki. Roztwór amoniaku efektywnie redukuje zanieczyszczenia bakteryjne ziarna kukurydzy. Zacier gorzelniczy ze zdezynfekowanego ziarna zawiera mniej niepożądanych mikroorganizmów niż zacier z ziarna nieodkażonego. Wyższa jest wydajność etanolu z ziarna odkażonego.
Wnioski. Roztwór amoniaku jest dobrym środkiem dezynfekującym do odkażania ziarna kukurydzy stosowanego jako surowiec do produkcji bioetanolu.

Słowa kluczowe: fermentacja etanolowa, zanieczyszczenia mikrobiologiczne, amoniak, ziarna zbóż

Accepted for print – Zaakceptowano do druku: 17.09.2009

For citation – Do cytowania: Broda M., Grajek W., 2009. Ammonia disinfection of corn grains intended for ethanol fermentation. Acta Sci. Pol., Technol. Aliment. 8(4), 33-38.