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# Integrated Process for Ammonia Inactivation of Aflatoxin-Contaminated Corn and Ethanol Fermentation

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A process is described for converting aflatoxin-contaminated corn to ethanol via combining ammonia inactivation with the liquefaction step of the ethanol fermentation process. Better ethanol yields were obtained when ammonia was added during liquefaction than when no ammonia was added. Aflatoxin $B_1$ levels were reduced 80 to 85% by the process.

A number of studies have been carried out on the fate of mycotoxins in contaminated grains used as substrates for the fermentative production of ethanol (6–11). Results common to these studies are: (i) little degradation of toxin during fermentation; (ii) no toxin in the distilled alcohol; and (iii) toxin accumulation in the spent grains. This last result presents a serious problem because the spent grains are often destined for animal feed. Consequently, practical detoxification procedures are essential.

Brekke et al. (3–5) developed a three-step gaseous ammonia process for the detoxification of aflatoxin-contaminated corn. Subsequently, we (11) reported that corn detoxified with the gaseous ammonia process can be used effectively for the production of ethanol and safe spent grains. However, this detoxification process is rather expensive, ranging from 26 cents/bu (4) to 66 cents/bu (G. E Hamerstrand, private communication, 1980), and is most effective at temperatures above 50°C and at an ammonia concentration of 1.5% (3). In a separate study, Lillehoj et el. (9) detoxified aflatoxin $B_1$ in wet postfermentation stillage with sodium hydroxide, ammonium hydroxide, sodium hypochlorite, and hydrogen peroxide. Substantial quantities of these chemical agents and high temperatures were required to achieve inactivation.

Therefore, since temperatures of approximately 90°C are commonly attained during the cooking step of the traditional ethanol process (11) and ammonia is useful both as a detoxifying agent for aflatoxin and as a nitrogen source for yeast (11), we undertook the research discussed in this note to develop a procedure for combining detoxification and fermentation into a single efficient integrated process.

First, we tested the feasibility of detoxifying aflatoxin-contaminated corn in an ammonia-corn slurry at 25°C and at a starch concentration (20 wt % solids) which approximates the slurry-liquefaction phases of the traditional ethanol fermentation process. The aflatoxin $B_1$ content (2) of a test lot of naturally contaminated corn obtained from Geogia was reduced 87% (617 μg/kg to 78 μg/kg) with 1.5% ammonia (weight of $NH_3$/weight of corn) after 4 days of reaction time. However, since a 4-day reaction time would be excessive as a prestep in a practical fermentation process and since ammonia detoxification is temperature sensitive (3), we decided to conduct a series of preliquefaction ammonia treatments at 64°C and to integrate these treatments with the traditional ethanol fermentation process described by Lillehoj et al. (9). Replicate fermentations were conducted in 2-liter Erlenmeyer flasks and in 20- and 50-liter fermentors with fermentation volumes of 1, 8, and 20 liters, respectively. The same lot of naturally contaminated corn, obtained from Georgia, was used in all tests. A mean aflatoxin $B_1$ destruction value of 89% was obtained with a 24-h, 1% (weight of $NH_3$/weight of corn) ammoniation treatment before liquefaction. The mean ethanol conversion efficiency after this pretreatment was 78%. However, when the ammoniation treatment was reduced to 2 h, 74% of aflatoxin $B_1$ was destroyed and ethanol conversion efficiency increased to 86%. Doubling the ammonia concentration to 2% (weight of $NH_3$/weight of corn) had little effect on detoxification and conversion efficiency. During control fermentations with no ammonia treatment, aflatoxin $B_1$ was concentrated (1,180 μg/kg) in the postfermentation solids, and ethanol conversion efficiency was 70% of the theoretical ethanol yield.

Although these aflatoxin destruction and conversion efficiency values were encouraging, we still sought to integrate ammonia detoxification more efficiently with ethanol fermentation. Thus we developed the integrated detoxification and fermentation process diagrammed in Fig. 1 and described by the sequential steps listed below

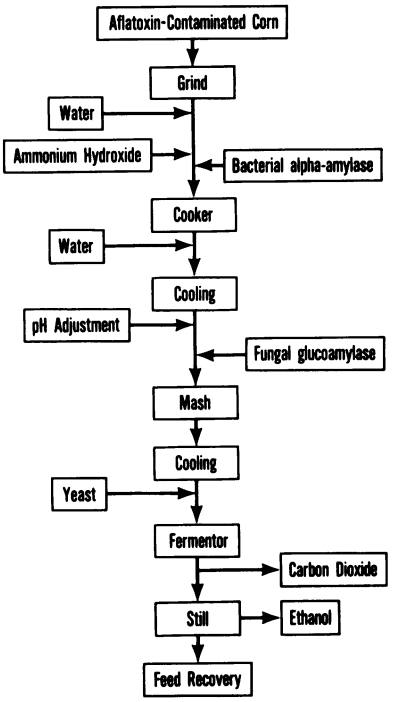


FIG. 1. Integrated detoxification and fermentation process.

for the fermentation of 1 bu of aflatoxin-contaminated corn.

**Detoxification and fermentation protocol for 1 bu of contaminated corn. (i) Grinding.** Aflatoxin-contaminated corn is ground into a fine meal to pass through a 10-mesh (2-mm) screen.

**(ii) Slurrying.** In the fermentation vessel, a mash (20% solids, wt/wt) is prepared by adding 23 gal (ca. 87.4 liters) of water to 56 lb (ca. 25.2 kg; 1 bu) of milled grain, followed by the addition of 1% ammonia as ammonium hydroxide based on the "as is" weight of the grain (i.e., approximately 2 lb [ca. 0.9 kg] of reagent ACS ammonium hydroxide per bu). Then a bacterial alpha-amylase (0.11 lb [ca. 49.9 g] per 56 lb of grain) is added for liquefaction. No pH adjustment is made.

**(iii) Liquefaction.** The alkaline mash (pH of approximately 9.5) is heated in a closed system with continuous agitation to a temperature of 90°C and held for 1 h.

**(iv) Conversion.** The mash is cooled to 60°C by addition of 6.2 gal (ca. 23.6 liters) of water, and the pH is adjusted to 4.2 with dilute hydrochloric acid. Fungal glucoamylase (0.4 lb [ca. 181.4 g] per 56 lb of grain) is added and the mash is held for 2 h at 60°C for conversion. The mash is then cooled to fermentation temperature (30°C), and the pH is adjusted to 4.5 with ammonium hydroxide.

**(v) Fermentation.** Distillers yeast (1%, vol/vol [0.3 gal/30 gal of mash]) is added. Before inoculation, the yeast is grown in YM medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1.0% glucose) for 24 h at 30°C. The inoculated mash is held at 30°C for 3 days as fermentation proceeds.

**(vi) Distillation.** The fermented mash is distilled in a pot or continuous still, and the alcohol is recovered.

**(vii) Feed recovery.** Recover the spent grains by filtration screening or centrifugation, and assay the wet (65 to 80% moisture) material for residual aflatoxin. If the toxin concentration is less than 100 μg/kg on a dry-matter basis, then a normal ration formulated for growing ruminants would contain less aflatoxin than the Food and Drug Administration feedstuff guideline of 20 μg/kg. However, if the aflatoxin content of the stillage exceeds this 100-μg/kg level, then a second ammonia detoxification treatment is necessary.

This protocol is based on experimental data (Table 1) obtained during 1- and 8-liter fermentations. An ammonia treatment of 1% (weight of $NH_3$/weight of corn) was more effective than 0.5% for aflatoxin destruction, whereas ethanol conversion efficiency was slightly better at the lower ammoniation level. Aflatoxin $B_1$ destruction and ethanol conversion efficiency were slightly better when the corn was ground to pass through a 10-mesh (2-mm) screen rather than a finer, 20-mesh (1-mm) screen. Most interesting was the observation that fermentations of aflatoxin-contaminated corn coupled with an ammonia treatment consistently produced more ethanol than fermentations of the same corn with no ammonia treatment. As noted previously (11), ammonia supplies needed nitrogen for the fermentative microorganism and should be included in any protocol for the fermentation of aflatoxin-contaminated corn. Inactivation of aflatoxin can simply be an additional benefit.

The primary difference between the fermentation process described above and the modified traditional ethanol fermentations discussed previously is that a bacterial alpha-amylase was used in place of barley malt during slurrying and liquefaction. The bacterial alpha-amylase has an optimum activity at 90°C and retains at least 25% activity at a pH of 10.0. Consequently, we were able to combine ammonia inactivation with liquefaction at 90°C for 1 h.

Last, we tested a second ammonia treatment to inactivate residual aflatoxin in wet spent grains recovered from the integrated process described in Fig. 1. After exposure to 1% ammonia (weight of $NH_3$/wet weight of spent grains)

TABLE 1. Combined detoxification and fermentation of aflatoxin-contaminated corn

| Ammonia concn[a] | Fermentation vol[b] (liters) | Grind size (mm) | Final ethanol concn by wt[c] (%) | Ethanol conversion efficiency[d] (%) | Aflatoxin $B_1$ (μg/kg) in dried spent grains[e] | Aflatoxin $B_1$ destruction[f] (%) |
|---|---|---|---|---|---|---|
| 0.5% ammonia | 1 | <2 | 5.8 | 83 | 300 | 75 |
| 1.0% ammonia | 1 | <2 | 5.8 | 83 | 183 | 85 |
| 0.5% ammonia | 1 | <1 | 5.4 | 77 | 366 | 70 |
| 1.0% ammonia | 1 | <1 | 5.2 | 74 | 182 | 85 |
| No-ammonia control | 1 | <2 | 4.3 | 61 | 1,205 | NA |
| 0.5% ammonia | 8 | <2 | 6.7 | 96 | 355 | 68 |
| 1.0% ammonia | 8 | <2 | 6.1 | 87 | 227 | 80 |
| No-ammonia control | 8 | <2 | 5.2 | 74 | 1,116 | NA |
| No-ammonia control (non-aflatoxin-contaminated corn) | 8 | <2 | 6.6 | 94 | ND | NA |

[a] Ammonia concentration (weight of $NH_3$/weight of corn) added during slurrying of corn naturally contaminated with aflatoxin (617 μg/kg).

[b] Replicate batch fermentations were conducted in 2-liter Erlenmeyer flasks described by Nofsinger and Bothast (11) and in 20-liter fermentors (Stainless Steel Products Co., St. Paul, Minn.).

[c] Samples were assayed for ethanol concentration on a Varian 3700 gas chomatograph equipped with a 6 ft (ca. 1.83-m) Porapak Q column operated at 190°C.

[d] Ethanol conversion efficiency is the assayed value of ethanol as a percentage of the theoretical (7%) ethanol yield by weight. The least significant difference for all replicated fermentations is 8.9. Conversion efficiencies differing by more than 8.9 are significant at the 0.05 level.

[e] Quantitative aflatoxin analyses were made by the Association of Official Analytical Chemists method recommended for corn. ND, None detected.

[f] Aflatoxin $B_1$ destruction is the difference between the assayed $B_1$ level in ammoniated spent grain and the $B_1$ level in non-ammoniated or control spent grain, as a percentage of the $B_1$ level in the control. The least significant difference for all replicated assays is 16.5. Aflatoxin destruction values differing by more than 16.5 are significant at the 0.05 level. NA, Not applicable.

for 24 h at 25°C, the aflatoxin content of the dried spent grains was reduced 80% (227 μg/kg to 46 μg/kg). This second ammonia treatment may be necessary because total aflatoxin destruction achieved via the process diagrammed in Fig. 1 is dependent on the original aflatoxin content of the corn, and one ammonia treatment may not be sufficient to produce spent grains low enough in aflatoxin content to be used safely in animal feed. A final aflatoxin content of 100 μg/kg in the spent grains would normally yield <20 μg/kg (the Food and Drug Administration guideline for aflatoxin [1]) in a formulated ration for growing ruminants.

Application and scale-up of the process described herein are currently under way in a cooperative-multidisciplinary project at the University of Illinois.

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