Source and Identification of Contamination of a Brewery Conveyor Lubricant

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Summary

On-site examination and sampling of a brewery conveyor line revealed that microbial species related to the degree of contamination of conveyor lubricant with beer. A contrived laboratory experiment involving mixtures of beer and lubricant confirmed the on-site findings regarding specific groups of microorganisms. Important dominant species included *Geotrichum* and anaerobic sulfate-reducing bacteria. Recommendations for control include preventive maintenance and housekeeping to reduce beer leakage and prevent slime build-up.

I. Introduction

The conveyor line in a brewery packaging operation is the last stop for the carefully prepared, quality assured product of the brewery. Its function is to facilitate the passage of empty cans to the fillers and from there to and through the pasteurizers. This considerable movement necessitates the use on the conveyor of a water-based lubricant with minimal foaming properties. The traditional components of such lubricants include alkaline salts of long-chain fatty acids, EDTA, nonionic surfactants, and (sometimes) alcohols. These lubricants are frequently diluted as much as 300:1 with water, thereby diluting and reducing any innate bioresistance of the product. The large open environment of the packaging operation and the irregular pattern of air movement combine to make absolute contamination control almost impossible. Even though there is only a remote chance for physical contact with the beer itself, any sights or smells developed in this area are not acceptable. The detection of slime on the conveyor with accompanying strong odors was the signal that initiated this study.

II. Materials and Methods

This study is divided into two parts:

1. The collection of environmental samples along the entire conveyor system emphasizing those sites of organoleptic concern, all of which were eventually assessed for pH and specific microbial flora.
2. The recreation of beer/conveyor lubricant mixtures representing conditions proximal and distal to the filling lines and subsequently challenging these mixtures with appropriate microbial inocula.

Collection of Environmental Samples

Fig. 1 gives the location and description of sample sites. Wherever possible, samples were collected in sufficient amount for laboratory analysis (Whirl-Pak Bags, Nasco). Where only exposed surfaces were available for sampling, Monitor and Monitor Y/M field tests (Diversey Wyandotte Corp.) were used for bacterial and fungal detection, respectively.

Organoleptic Observation

At every sampling site, the area was examined for the presence of microbial slime, color, and odor on and around the slime deposits.

Microbial Quantitative and Qualitative Analysis

Standard microbiological methods were used for the counting and differentiation of the various groups detected. Isolation of microbial groups was made on the following media:

Tryptic soy agar (Difco Laboratories, Detroit, MI)
Potato dextrose agar (Difco Laboratories, Detroit, MI)
MacConkey agar (Difco Laboratories, Detroit, MI)
Wort agar (Difco Laboratories, Detroit, MI)
Inhibitory mold agar (Gibco Laboratories, Grand Island, NY)
Universal beer agar (Gibco Laboratories, Grand Island, NY)
WL Differential agar (brewery organisms) (Gibco Laboratories, Grand Island, NY)
SR Deep kit (sulfate-reducing bacteria) (Biosan Laboratories, Inc., Ferndale, MI)

Identification of bacteria and fungi was based on microscopic examination and commercial biochemical test systems (API 20 C, API 20 F - API, Analytab Products; and Rapid NFT - DMS Laboratories, Inc.) when appropriate. All incubation was at 28°C for a minimum of 48 hours and up to 7 days for fungi and sulfate reducing bacteria.

1. On-site collected liquid samples were transported in an ice chest to the laboratory and worked up within 24 hours. Sites sampled with Monitor and Monitor Y/M were incubated immediately at ambient temperatures and examined 24 hours later in the laboratory.
2. On the basis of the results of No. 1 above (Table 1), a contrived experiment involving various beer/lubricant mixtures was set up:

Sample:	1	2	3	4	5	6	7	8	9	10	11	12
% Beer	100	90	80	70	60	50	40	30	20	10	0	PO_4
% Lubricant	0	10	20	30	40	50	60	70	80	90	100	buffer

These were inoculated at a final dilution of 1:100 with slime containing a diverse and high population (see Fig. 1, Site 4). The microbial assessment was done at 0 time in the PO_4 buffer (pH 7.0) and at 24 hours and 5 days in all 12 samples.

III. Results and Discussion

The samples taken on-site from various locations of the conveyor line indicate that the problems related to the lubricant are due to contaminants attracted to the beer/lubricant mixture (Table 2). The contribution

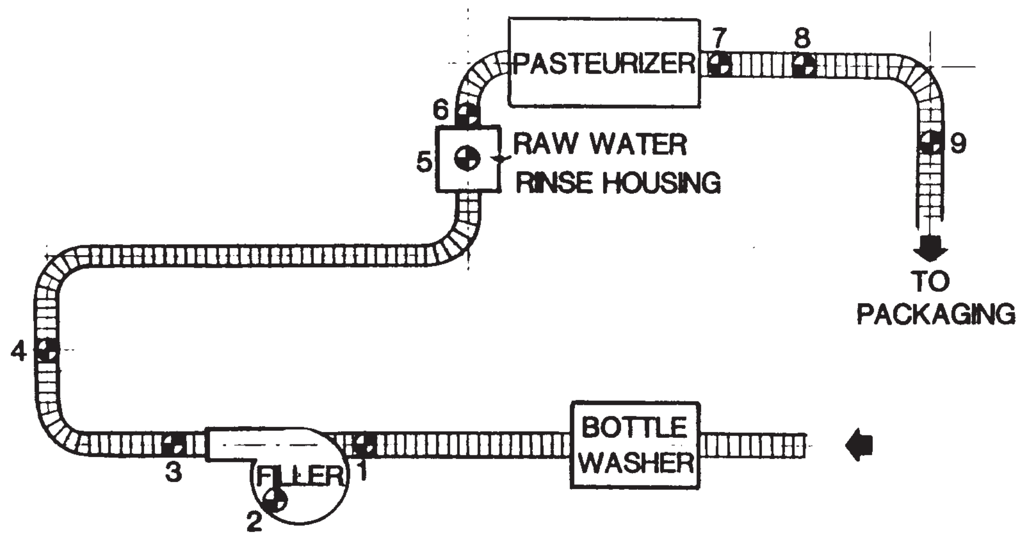

Fig. 1 Schematic diagram of conveyor system with sampling sites.

KEY: **Site 1** (before filler) – sampled lube spray site/lube conveyor chain surface using Whirl-Pak Bag, M, and Y/M.
Site 2 (filler) – sampled beer/lube mixture dripping.
Site 3 (after filler) – sampled lube spray site/lube chain under tray/surface wipe using Whirl-Pak Bag, M, and Y/M.
Site 4 (conveyor curve) – sampled slime/beer/lube mix using Whirl-Pak Bag.
Site 5 (rinse water) – sampled water using Whirl-Pak Bag.
Site 6 (before pasteurizer) – surface wipe sample using M and Y/M.
Site 7 (after pasteurizer) – surface wipe sample using M and Y/M.
Site 8 (conveyor idle) – surface wipe sample using M and Y/M.
Site 9 (before packaging) – surface wipe sample using M and Y/M.

of leaking beer to the contamination problem is two-fold. It lowers the pH of the conveyor lubricant and adds more readily usable nutrients for microbial growth. The dominant organisms around the filler area are primarily *Saccharomyces* species (Lodder 1970). The finding of some wild yeasts among the contaminants should be of sufficient seriousness by itself to promote more attention to this problem. As samples were taken more distant from the filler, the dominant microbial flora shifted to bacteria accompanied by *Geotrichum candidum*. In fact, *Geotrichum* was found in sufficient levels to produce a fetid odor along the sampling site. *Geotrichum* is an indicator organism of plant insanitation (Eisenberg & Cichowicz 1977).

The results with the contrived mixtures concur with the results found in on-site sampling. Table 2 shows *Geotrichum* replacing *Saccharomyces* as the percentage of beer in the beer/lubricant mixture decreases.

The dominant aerobic bacterial groups found belong to *Pseudomonas* and *Klebsiella* genera. Neither of these findings is surprising. *Pseudomonas* is the dominant group in every industrial process water system (Richards 1975), whereas *Klebsiella* is a ubiquitous member of high BOD waste systems including those from breweries and food processing plants (Brown & Seidler 1973).

Aerobic and facultative bacterial species were not followed in the contrived experiment since *Pseudomonas* and *Klebsiella* isolates were found throughout the conveyor operation.

A surprising and disturbing finding was associated with slime deposits that were blackened or greyish. These were positive for a group of bacteria referred to as anaerobic sulfate reducing bacteria (Postgate 1979). They grow only in the absence of molecular oxygen and reduce sulfate to sulfide. The H_2S is responsible for the rotten egg smell associated with this organism. Just as important is the possibility of

Table 1 Quantitative and qualitative evaluation of chain conveyor samples, bottling line 50.

Sample	Location (see Fig. 1)	Organoleptic evaluation	pH	*CFU/ml or monitor estimates	Identification
A. Surface wipe before filler (before lube spray head)	1	No slime build-up could be observed	N.D.	$B = \geq 10^5$ $YM = \geq 10^3$	*Geotrichum* sp. *Pseudomonas* sp.
B. Lube from spray head (1:300 dilution)	1	Color: Clear Odor: None	8.6	$B = \geq 10^1$ YM=None	*Pseudomonas* sp.
C. Beer dripping (at filler)	2	Color: Amber Odor: Beer	6.5	$B = 1 \times 10^2$ $M = 1 \times 10^3$ $Y = 1 \times 10^4$	*Lactobacillus* sp., *Hafnia* sp. *Pediococcus* sp. **Klebsiella* sp. *Desulfovibrio* sp. **Saccharomyces* sp. *Geotrichum* sp. *Alternaria* sp.
D. Slime after filler (under lube spray head)	3	Color: Brownish black Odor: Acidic fruity	9.0	$B = 7 \times 10^2$ $M = 4 \times 10^3$ $Y = 5 \times 10^5$	*Klebsiella* sp. *Desulfovibrio* sp. **Saccharomyces* sp., *Geotrichum* sp.
E. Lube after filler from sprayhead (1:100 dilution)	4	Color: Clear Odor: Not detected	9.4	B=None M=None Y=None	Sample is free of contamination
F. Slime at curve (after filler)	4	Color: Chalky gray Odor: Rotten egg (strongest odor at this location)	6.5–7.0	$B = 3 \times 10^8$ $M = 1 \times 10^4$ $Y = 2 \times 10^3$	**Klebsiella* sp., **Pseudomonas* sp. **Desulfovibrio* sp., *Saccharomyces* sp. **Geotrichum* sp., *Citrobacter* sp.
G. Rinse water at "Doghouse"	5	Appearance: Clear Odor: Not detected	7.8		**Geotrichum* sp. *Pseudomonas* sp., *Flavobacterium* sp.
H. Slime after raw water rinse	6	Color: Dark gray Odor: Rotten egg (friable slime)	N.D.	$B = \geq 10^5$ $YM = \geq 10^3$	*Klebsiella*, *Desulfovibrio* sp. **Geotrichum* sp., *Sarcina lutea*
I. Slime after pasteurizer ("Late side")	7	Color: Not detected Odor: Slight	N.D.	$B = \geq 10^1$ $YM = \geq 10^1$	Not done (reduced contamination on chain conveyor right after pasteurizer discharge)
J. Slime conveyor idle	8	Color: Black Odor: Rotten egg	N.D.	$B = \geq 10^5$ $YM = \geq 10^3$	**Geotrichum* sp., *Pseudomonas* sp. **Desulfovibrio* sp., *Klebsiella* sp.
K. Conveyor surface wipe before packaging area	9	—	N.D.	$B = \geq 10^5$ $YM = \geq 10^3$	*Geotrichum* sp. *Pseudomonas* sp.

*CFU=Colony Forming Unit/Milliliter Sample (organism/ml)
N.D.=Not Done
B=Bacteria
Y=Yeast
M=Mold
The dominant species in a mixed population are starred.

Table 2 Survival of slime microorganisms in beer/lubricant mixtures.

				Colony Forming Unit/ml					
				1 day		5 days		Sulfate Reducers	
Beer %	Lube %	pH	0 Day	B	F	B	F	1 day	5 days
100	0	4.1	1×10^7B; 4×10^6F	2×10^6	1×10^6	1.6×10^5	4.1×10^5(S)	+	–
90	10	4.3		1.3×10^7	5×10^6	2.4×10^5	4.8×10^5(S)	+	–
80	20	4.5		1.7×10^7	1×10^6	1.2×10^7	7.6×10^6(S)	+	–
70	30	4.6		2×10^7	3×10^5	2.2×10^7	2.5×10^6(S)	+	–
60	40	4.8		6.2×10^7	2×10^5	4.1×10^7	5×10^7(G)	+	–
50	50	5.2		1.2×10^8	6×10^5	6.8×10^7	8.5×10^7(G)	+	–
40	60	5.5		1.3×10^8	8×10^5	6.8×10^7	2×10^8(G)	+	–
30	70	5.6		1.5×10^8	8×10^5	4.3×10^7	3×10^7(G)	+	–
20	80	6.2		1.6×10^8	7×10^5	2.3×10^7	1×10^7(G)	+	–
10	90	6.9		1.4×10^8	6×10^5	1.1×10^7	1.6×10^7(G)	+	–
0	100	9.7		7.3×10^7	4×10^5	2.5×10^8	5.6×10^5(G)	+	–
PO_4 Buffer		7.0		3.9×10^7	8×10^5	5×10^7	$<10^4$	+	+

B=Bacteria Y=Yeast M=Mold F=Fungi Y+M=Yeast and Mold mixed culture
+=Survival –=No survival
Note: At the 60/40 mixture the fungal/yeast population shifted from *Saccharomyces* dominance to *Geotrichum* dominance with the former disappearing in the 0/100 mixture.

corrosion of ferrous metals by this sulfate reduction process. The survival studies done with the contrived series (Table 2) show that after 24 hours all systems had sufficient sulfate-reducing bacteria to produce a positive test. However, after five days, only the PO_4-buffer allowed survival. If the system is not continuously reinoculated, neither the beer nor lube will support this organism.

The results indicate that beer/lubricant mixtures provide nutritional conditions for a variety of organisms derived from the environment. Optimal control of this problem may be difficult due to inherent shortcomings of the conveyor system design itself, which permits collection of beer leaks with poor drainage and contains areas not easily reachable for clean-up. Adequate maintenance to control beer leaks around the filler and good housekeeping to prevent the slime build-up is a necessity. Slime and odor problems do not appear immediately after a cleaning operation but develop rapidly after about three days of conveyor operation. Regular and strict housekeeping regimes will be needed to deter the development of the problem described here.

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