Journal of Industrial Microbiology & Biotechnology (2000) 25, 86–92

www.nature.com/jim

Foam control in biopesticide production from sewage sludge

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Several antifoam agents were evaluated for the ability to control foam in the production of *Bacillus thuringiensis*-based biopesticides using sewage sludge as a raw material. Experiments were conducted in shake flasks as well as in 15 l fermentors with controlled parameters. Polypropylene glycol (PPG), the most commonly used antifoam agent in *B. thuringiensis* fermentation, inhibited cell growth, sporulation and decreased the entomotoxicity yield even at a concentration of 0.1% (v/v) in sewage sludge medium. About 40% reduction in entomotoxicity was observed when PPG was used at 0.3% (v/v). The impact of PPG on sporulation and toxin synthesis in tryptic soy yeast broth (TSYB) medium was also studied. The inhibitory effects were less severe in TSYB than in sludge medium. Another silicone-based antifoam agent, ''Antifoam A'', showed less severe effect on growth and stendotoxin production. The problem of the inhibitory effect of chemical antifoam agents on growth and endotoxin production was minimised substantially with the use of vegetable oils such as canola, olive, and peanut oils. Canola and peanut oil stimulated both sporulation and δ-endotoxin synthesis. The stimulus effect varies with the monounsaturated fat contents of oils. *Journal of Industrial Microbiology & Biotechnology* (2000) **25,** 86–92.

Keywords: foam control; biopesticide production; *Bacillus thuringiensis*; entomotoxicity; δ-endotoxin; spores; *Choristoneura fumiferana*; spruce budworm; natural oils; sewage sludge

Introduction

In addition to aeration, agitation and other hydrodynamic conditions, the protein content of the microbial growth medium is the most vulnerable factor for foam formation in fermentation systems. Intensive foam formation frequently engenders productivity losses such as reduction in working volume of a bioreactor [8], product and biomass losses, cell lysis, enhanced gas hold up, decreased power dissipation and liquid circulation rate, lower mass and heat transfer and increased chances of contamination during fermentation [18]. The concentration of components initially present in the medium and products of biochemical reactions as well as operating conditions significantly influenced the stability and pattern of foam formation [20]. Foam control needs more attention when sewage sludge is used as a sole medium due to the fact that sludge is composed of approximately 95% bacterial cell mass (dry sludge basis) and is rich in proteins.

Foam formation in biopesticide fermentation is controlled mainly by the addition of polypropylene glycol (PPG) and silicone-based antifoam agents [5,10]. These antifoam agents affect the respiratory activity by affecting transport of nutrients through the cell walls, including oxygen transport. They influence the physiology of cells and cells grown in the presence of these agents resemble physiologically those grown under oxygen limitation [20]. High aeration rates are necessary to maximize cell growth, sporulation and δ-endotoxin production by *Bacillus thuringiensis* (Bt) [3,5,13], and causes severe foaming in fermentation processes. The dissolved oxygen concentration in the fermentation liquid was also correlated with cell respiration rates [9] and δ-endotoxin synthesis by Bt [1].

Many problems associated with chemical antifoam agents can be eliminated with the use of natural oils which, apart from controlling foam [17,18], work as an oxygen vector [12]. The selection of an antifoam agent depends on its ability to suppress foam, volume required and unit cost of antifoam. This report deals with the selection of the best antifoam agent based on overall efficiency of foam suppression in Bt fermentation using sewage sludge as a raw material.

Materials and methods

Bacterial strain

B. thuringiensis var. *kurstaki* HD-1 (ATCC 33679) was used. It was subcultured and streaked on tryptic soy agar plates [TSA: 3.0% Tryptic Soy Broth (Difco, Becton Dickinson & Company, Sparks, MD, USA)+1.5% Bacto-Agar (Difco, Becton Dickinson & Company, Sparks, MD, USA)], incubated for 24 h at 30±1°C and preserved at 4°C for future use.

Antifoam agents

The antifoam agents used in this study include "Antifoam A" (Sigma Chemicals, St. Louis, MO, USA), PPG (Aldrich Chemical Company, Milwaukee, WI, USA) and most common natural oils available in the market. The natural oils used were canola oil (Crisco brand, Proctor and Gamble Inc., Toronto, Ontario, Canada), corn oil (President Choice, Sun Fresh Ltd., Toronto, Ontario, Canada), olive oil, peanut oil, soybean oil (all of Generation, Minix Inc., Montreal, Québec, Canada), sesame (China Sun, National Importers Ltd., New Westminster, BC, Canada) and sunflower oil (Safflo, Unico Inc.). All

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Received 9 February 2000; accepted 6 June 2000

Table 1 Physico-chemical and biological characteristics of secondary sludge (CUQ)

Characteristics	Quantity
Physical characteristics	
Total solids (g/l)	29
Volatile solids (g/l)	20
Suspended solids (g/l)	24
Volatile suspended solids (g/l)	18.5
pH	5.8
Chemical characteristics	
Total carbon (mg/kg)	459,925
Total nitrogen (mg/kg)	44,080
Ammonical nitrogen (mg/kg)	793
Nitrogen as NO_x (mg/kg)	17
Total phosphorus (mg/kg)	20,846
Phosphorous as PO_4 (mg/kg)	12,549
Sulfur	7685
Metals (mg/kg)	
Al	12,918
Ca	18,859
Cd	0.9
Cr	128
Cu	297
Fe	15,050
K	3856
Pb	31
Zn	386
Na	4806
Biological characteristics	
Total plate count (cfu/ml)	1.20 E+10
Total coliform bacteria (cfu/ml)	1.00 E+07
Fecal coliform (cfu/ml)	1.60 E+06
Fecal streptococci (cfu/ml)	3.20 E+05

antifoam agents except "Antifoam A" were used as 1:5 diluted emulsion with water in the bioreactor. "Antifoam A" was used as an emulsion at a higher dilution (1:10) as recommended by the manufacturer.

Sludge samples

Secondary sludge from Communauté Urbaine de Québec (CUQ) wastewater treatment plant was used in this study. The secondary sludge was decanted to increase sludge solids concentration. The decanted sludge was homogenised and pasteurised in a 150 l fermentor in order to keep a constant quality of the sludge. In order to reduce sludge deterioration during storage, the pasteurised sludge was stored at 4°C until used. The sludge, unless otherwise mentioned, was used at a solids concentration of 25 g/l (dry weight). Table 1 presents the composition of the sludge. Sludge was sterilised at 121°C for 30 min and its pH was adjusted to 6.9±1 with 2 N NaOH after sterilisation.

Inoculum and culture conditions

A loopful of bacterial growth from a TSA plate was used to inoculate a 500-ml Erlenmeyer flask containing 100 ml of tryptic soy yeast broth (TSYB: 3.0% tryptic soy broth+0.3% yeast extract; Difco, Becton Dickinson & Company, Sparks, MD, USA). Before inoculation, TSYB medium was sterilised at 121°C for 15 min. The flask was incubated on a rotary shaker at 300 rpm and 30°C for 10–12 h. A 2% (v/v) inoculum from this flask was then used to inoculate 500 ml Erlenmeyer flasks containing 100 ml sterilised sewage sludge. The flasks were incubated in the same way for 12 h. These actively growing cells were used as inoculum for both shake flasks and a 15 l fermentor (Biogenie Inc., Québec, Canada).

Fermentation procedure

Shake flask experiment: A 2.0% (v/v) inoculum was used to inoculate 500 ml Erlenmeyer flasks containing 100 ml sterilised TSYB medium or sewage sludge. PPG (0–0.3%, v/v) was incorporated in TSYB and sludge media to study the inhibition of PPG on cell growth and δ-endotoxin production. In another experiment, natural oils were incorporated at 0.4% (v/v) in sludge medium to study their impacts on cell yield, spore yield and entomotoxicity. Flasks were incubated on a rotary shaker at 300 rpm for 48 h. At intervals, samples were withdrawn for analysis of viable cell count (VC), spore count (SC) and entomotoxicity.

Fermentor: Fermentors (15 l capacity, with dissolved oxygen and pH probes) containing 10 l of sewage sludge were sterilised *in situ* at 121°C for 30 min. A 2.0% (v/v) inoculum was used. Temperature and pH of the fermentation liquid were controlled at 30°C and 6.9±0.1, respectively. The fermentor contents were agitated at 400 rpm. Airflow rate was controlled automatically using a computer-controlled system in order to maintain dissolved oxygen above 25% saturation. At intervals, samples were withdrawn aseptically for analysis of VC, SC and entomotoxicity and to check purity of the culture.

Analytical

Samples were analysed for VCs and SCs as colony-forming units (cfu) by serial plating technique on TSA. Appropriately diluted samples were plated and incubated overnight at 30°C. For SCs, appropriately diluted samples were subjected to 75°C for 10 min before plating them. The entomotoxicity of the samples was measured by bioassay as a relative mortality of

Table 2 Composition of the spruce budworm diet

Ingredient	Quantity
Casein, vitamin free	35 g
4 M Potassium hydroxide	5 ml
Cellulose powder (Alphacel, Nutritional Biochemicals Corporation, Cleveland, OH, USA)	5 g
Salt mixture (Wesson, Nutritional Biochemicals Corporation, Cleveland, OH, USA)	10 g
Sucrose	35 g
Wheat germ	45 g
Choline chloride	1 g
Vitamin solution[1]	10 ml
Ascorbic acid	4.0 g
Formalin (37% formaldehyde)	0.5 ml
Methyl paraben	1.5 g
Aureomycin powder (chlortetracycline hydrochloride, 55 mg/g)	5.5 g
Agar	17 g
Distilled water (volume made up to)	1000 ml

[1]Vitamin solution contains (mg/100 ml): niacin 100, calcium pantothenate 100, riboflavin 50, thiamine hydrochloride 25, pyridoxine hydrochloride 25, folic acid 25, biotin 2 and vitamin B_{12} 0.2.

spruce budworm larvae in comparison to the mortality caused by the standard preparation of Foray 48B (Abbott Laboratories, Chicago, IL, USA).

Bioassays

Bioassays were carried out using third instar larvae of eastern spruce budworm, *Choristoneura fumiferana* kindly supplied by Laurentian Forestry Centre, Sainte-Foy, Canada. Larvae were reared on a semi-synthetic diet (Table 2) containing formaldehyde and potassium hydroxide and by using a *diet incorporation method* [2]. In this method, 1.0 ml of serially decimal diluted sample was mixed with 20 ml liquid agar (usually 55°C)-based diet and distributed into 20 flat bottom glass vials (1 ml in each vial). After the mixture had solidified, one larva (third instar) was placed in each vial. The vials were covered with perforated plastic caps and incubated at ambient temperature for 5–7 days under a 40 W light bulb. Three dilutions of each sample and a standard were used in each bioassay. Three sets of control (without Bt preparation, sterilised sludge and water) were included in the procedure to correct the mortality of larvae due to the diet only. If a larval mortality of more than 10% was observed in controls, the whole bioassay was repeated. The entomotoxicity of the Bt preparation was calculated as mortality observed relative to the standard preparation and expressed in terms of international units of toxicity per microliter (IU/μl). The choice of this bioassay procedure was due to better homogenicity of the Bt samples throughout the diet.

The entomotoxicity of the Bt preparation was calculated by dividing the mortality of spruce budworm larvae expressed in the preparation by that of the Foray 48 B (48×10^9 IU/gal) and multiplying it with the toxicity of the Foray 48B (12600 IU/μl).

Efficiency parameters

Volumetric antifoam fraction (ε_v), cost coefficient (C_C), productivity coefficient (P_C), foam suppression coefficient (FS_C) and efficiency coefficient (E_C) are the dimensionless group and are defined by Equations 1–5:

$$\varepsilon_v = V_A/V_L \tag{1}$$

where V_A=total volume of an antifoam agent used to control the foam during fermentation, V_L=total medium volume in the fermentor:

$$C_C = C_A/C_{PPG} \tag{2}$$

where C_A=unit cost of an antifoam agent (\$/1), C_{PPG}=unit cost of PPG (\$/1):

$$P_C = ET_A/ET_{PPG} \tag{3}$$

where ET_A=entomotoxicity obtained using an antifoam agent (IU/μl), ET_{PPG}=entomotoxicity obtained using PPG as antifoam agent (IU/μl) and:

$$FS_C = (h_f - h_{f\,min})/h_f \tag{4}$$

where h_f=average foam height before addition of antifoam agent, $h_{f\,min}$=minimum (residual) foam height after addition of antifoam agents.

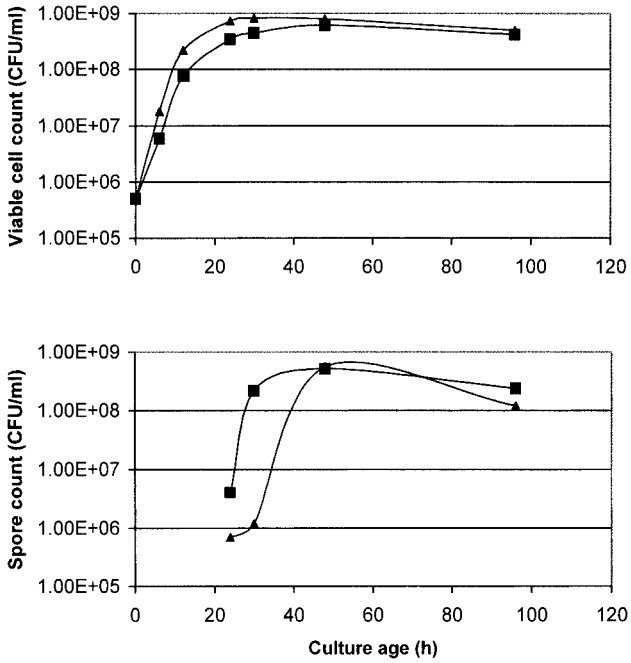

Figure 1 VC and SC for Bt; (▲) TSYB and (■) sludge medium.

The efficiency coefficient (E_C) of an antifoam agent is a cumulative effect of three parameters (Equation 5):

$$E_C = FS_C/(\varepsilon_v C_C) \tag{5}$$

Results

Shake flask studies

Figure 1 shows the pattern of VC and SC with the progress of fermentation in TSYB and sludge medium when actively grown inoculum (prepared in the respective media) was transferred into the flasks at a 2% (v/v) level. The viable cells multiplied exponentially in both media (Figure 1). The spore concentration in sludge was high from the beginning and can be attributed to some nutrient limitations. It is important to note that the sludge is composed of various nutrient components; easily biodegradable, less biodegradable, hard to biodegrade and non-biodegradable organic components [4]. The quantity of easily biodegradable matter (most likely to be consumed by Bt) in the sludge medium is normally low compared to TSYB. This could cause faster sporulation of cells in the sludge medium.

A decrease in cell mass yield was observed even at a lower volume fraction of PPG (0.1% v/v) used in the sludge medium (Figure 2). The spore concentration, as well as entomotoxicity at the end of the fermentation cycle, was severely affected by the addition of PPG and the effect varied proportionally with the PPG volume fraction used. A loss of 30% entomotoxicity was observed when cells were cultured with 0.3% (v/v) or $0.003\varepsilon_v$ of PPG (Figure 2). PPG reduced the cell yield even at lower volume fraction when cells were grown in rich medium (TSYB medium) (Figure 3). However, PPG's adverse effect on sporulation and entomotoxicity in TSYB was less severe than observed in the sludge medium. Higher sporulation in sludge

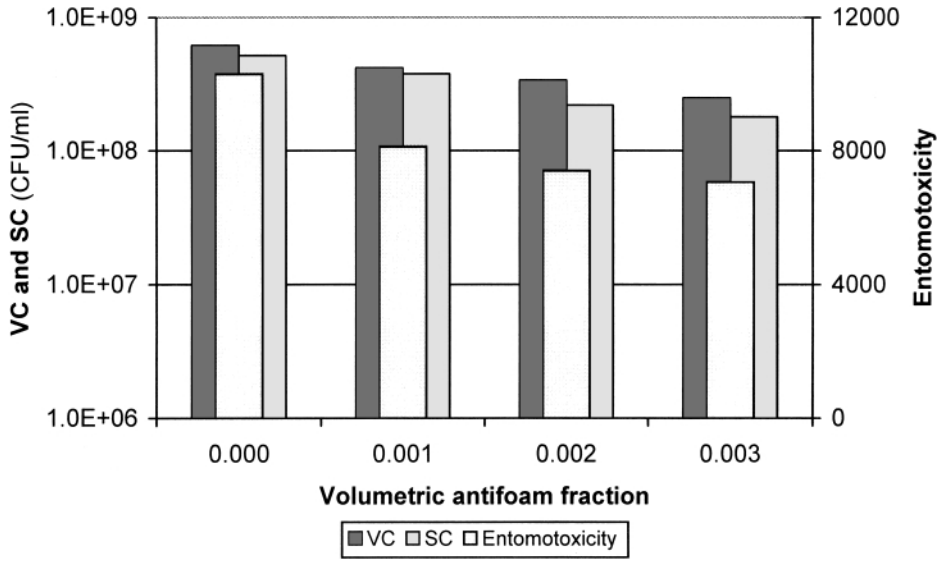

Figure 2 Effect of PPG on VC, SC and entomotoxicity of Bt grown in sludge medium in shake flasks.

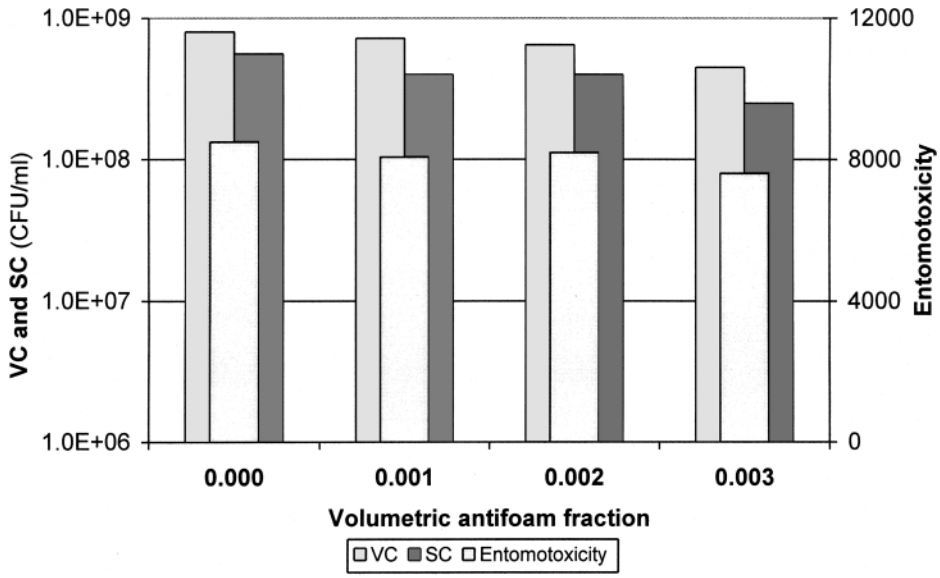

Figure 3 Effect of PPG on VC, SC and entomotoxicity of Bt grown in TSYB medium in shake flasks.

Table 3 Comparative impact of PPG on entomotoxicity in TSYB and sludge medium

PPG concentration percent (v/v)	TSYB medium				Sludge medium			
	VC (cfu/ml)	Percent sporulation[a]	Specific entomotoxicity (IU/spore · 10^{-3})	Relative entomotoxicity[b]	VC (cfu/ml)	Percent sporulation[a]	Specific entomotoxicity (IU/spore · 10^{-3})	Relative entomotoxicity[b]
0.00	8.0 E+08	70.0	15.15	1.000	6.2 E+08	83.9	19.82	1.000
0.10	7.2 E+08	55.6	20.16	0.950	4.2 E+08	90.5	21.33	0.787
0.20	6.5 E+08	61.5	20.46	0.965	3.4 E+08	64.7	33.60	0.717
0.30	4.5 E+08	55.6	30.41	0.896	2.5 E+08	72.0	39.20	0.685

[a]Percent sporulation represents the percentage of SC relative to VC.
[b]Absolute values for entomotoxicity in TSYB and sludge medium are 8484 and 10,304 IU/μl.

Table 4 Evaluation of oils as antifoam agents at 0.4% (v/v) in shake flasks

Oil	Cell yield (cfu/ml)[a]	Percent sporulation[a,b]	Entomotoxicity (IU/μl)
None	3.6×10^8	66.6	10,206
Canola	4.1×10^8	80.2	12,012
Corn	3.1×10^8	72.0	9786
Peanut	3.3×10^8	90.1	12,030
Olive	3.7×10^8	78.4	11,718
Sesame	3.1×10^8	80.6	10,206
Soybean	3.0×10^8	60.0	10,248
Sunflower	3.0×10^8	51.7	9534

[a]The values shown for cell yield and percent sporulation were an average of three data sets.
[b]Percent sporulation represents the percentage of SC relative to VC. Standard deviations for VC and SC were 8.0% and 7.6%, respectively. Standard deviation for entomotoxicity was also of the same order.

than TSYB medium was observed at a given concentration of PPG (Table 3).

There was an initial increase in sporulation with PPG concentration (0–0.1% PPG) followed by a decrease in sporulation (0.2% and 0.3% PPG concentration) (Table 3) when sludge was used as a production medium. However, a decrease in sporulation with PPG concentration was observed using TSYB as production medium. The difference in sporulation observed in TSYB medium and sludge medium with PPG concentration may be due to the foam characteristics and type of foam generated by dissolved substances as well as to the presence of microorganisms in the fermentation liquid and their dispersity in the media [19].

Corn, canola, olive, peanut, sesame, soybean and sunflower oils were evaluated for their impact on cell, spore and toxin yield in shake flask experiments before switching to the bioreactor. All the natural oils tested, except corn and sunflower oils, imparted no adverse effect on sporulation and toxicity yield. The cell yield and spore yield were similar with and without oil (control). In case of soybean oil, sporulation was decreased without affecting entomotoxicity as compared to control (Table 4). Relatively high VC, SC and entomotoxicity yield with canola, olive and peanut oils (Table 4) may be due to high monounsaturated fat contents and low polyunsaturated fat contents (Table 5). Higher polyunsaturated fat contents lower the yield of viable cells and entomotoxicity. Toxin synthesis and sporulation were not affected by sesame oil. The slight lower entomotoxicity observed in olive oil compared to canola and peanut oils demonstrates that the high monounsaturated fat content is not the only factor responsible for higher entomotoxicity. To ensure high productivity, a good balance in mono- and polyunsaturated fat content of oil is essential because the unsaturated fat could be utilised by the organisms [11]. Although the saturated fat content of the oils did not seem to affect either cell or toxin yields, a lower concentration (less than its polyunsaturated fat content) may be necessary for the better control of foam during fermentation.

Table 6 Foam suppression coefficient of antifoam agent

Antifoam agent	Foam height[a], h_f (cm)	Minimum foam height, $h_{f\,min}$ (cm)	Foam suppression coefficient, FS_C
PPG	11.76	0.4	0.966
"Antifoam A"	11.16	0.2	0.982
Canola oil	10.96	0.6	0.945
Peanut oil	9.76	0.6	0.939
Olive oil	8.92	0.4	0.955
Soybean oil	10.64	0.8	0.925

[a]The h_f value shown is an average of five sets of data.

Fermentor studies

The natural oils, "Antifoam A" and PPG, were tested for foam control in a 15 l fermentor. Table 6 presents the foam suppression efficiency (FS_C) of the antifoam agents. Antifoam A had shown the best foam suppression followed by PPG. All the natural oils tested had shown good foam suppression capabilities, but were inferior to chemical antifoam agents. The results obtained with all the oils (except soybean oil) in the fermentor (Table 7) were similar to that observed in shake flasks (Table 4). Soybean oil gave better results in the fermentor. This may be due to differences in rheological properties (mixing pattern, turbulence, *etc.*) of the medium contained in shake flasks and in fermentors. Peanut and canola oils were better antifoam agents with respect to toxicity values observed in the fermentor using sewage sludge (Table 7). These results are similar to those obtained in shake flasks. The severe foam formation was observed in sludge medium during the spore and endotoxin production phase of Bt fermentation. This observation resembles that reported by other workers [20] using synthetic medium.

Compared to PPG, "Antifoam A" showed better foam suppression (Table 6) with a less severe effect on VC and toxin yield compared to PPG in sludge medium (Table 7). The time required to suppress the foam was also shorter. This can ensure additional safety for the delayed response of peristaltic pumps used for antifoam addition for fast-growing foam. This could also allow

Table 5 Composition and cost of oils used

Oil	Fat content (g/g)	Saturated fat (g/g)	Monounsaturated (g/g)	Polyunsaturated (g/g)	Cost ($/l)
Canola	0.92	0.07	0.58	0.27	5.69
Corn	1.00	0.12	0.32	0.56	2.99
Peanut	1.00	0.17	0.51	0.32	3.49
Olive	0.96	0.13	0.73	0.10	6.98
Sesame	Data not available				25.27
Soybean	1.00	0.15	0.20	0.61	2.98
Sunflower	0.92	0.12	0.15	0.65	2.99

Unit cost of PPG and "Antifoam A" concentrate are 102.2 and 408.0 ($/l), respectively.

Table 7 Feasibility of antifoam agents in fermentor

Antifoam agent	Volumetric antifoam fraction, ε_v	Entomotoxicity (IU/μl)	Cost coefficient, C_C	Productivity coefficient, P_C	Efficiency coefficient, E_C	Spore concentration (cfu/ml)	Percent sporulation
PPG	0.0035	10,374	1.000	1.000	276	1.62 E+08	72.6
Antifoam A	0.0020	11,690	3.992	1.127	123	3.50 E+08	85.4
Canola oil	0.0045	12,348	0.055	1.190	3775	3.75 E+08	83.3
Peanut oil	0.0050	13,020	0.034	1.255	5497	3.90 E+08	83.0
Olive oil	0.0040	11,946	0.068	1.152	3496	3.57 E+08	81.1
Soybean oil	0.0055	11,886	0.029	1.146	5747	3.00 E+08	83.3

the foam to rise for a longer time before being destroyed by a chemical antifoam agent.

The choice of an antifoam agent is generally determined by three factors: the volume required for foam control should be minimum; it should increase the product yield; and fast destruction of foam by the antifoam agent is highly desirable. In order to evaluate the performance of antifoam agents, the foam suppression coefficient (FS_C) (Table 6), cost coefficient (C_C), volumetric antifoam fraction (ε_V), productivity coefficient (P_C) and efficiency coefficient (E_C) of antifoam agents (Table 7) were calculated from the results with the fermentor. The results suggest that "Antifoam A" comparatively requires minimum volume (lowest volumetric antifoam fraction) to control the foam during fermentation followed by PPG (Table 7). However, the cost coefficient of "Antifoam A" is four times higher compared to PPG. All the natural oils tested in fermentor are more cost-effective than chemical antifoam agents. C_C values of natural oils are at least 15 times less compared to PPG. The productivity coefficient of all the oils used is superior to PPG.

The efficiency coefficient, E_C, of natural oils was higher than that of chemical antifoam agents. Peanut and soybean oils were 20 times more efficient than PPG in foam suppression. These oils have at least 45 times higher E_C values than "Antifoam A". "Antifoam A" showed less severe effects on the spore yield and toxin yield of Bt. Thus, peanut and soybean oils appear to be the most favourable. However, the entomotoxicity obtained in sludge fermentation using soybean oil was much lower than that observed in peanut oil.

Discussion

In order to develop a feasible technology for biopesticide production, our principal objective is to incorporate a cheap raw material, preferably wastes generated at wastewater treatment plants (wastewater sludge). Various agricultural and industrial byproducts have been investigated for biopesticide production including citrus peels, wheat bran, corn meal, seeds of dates, beef blood, silkworm pupal skin, ground nut cake, cane molasses, fish meal, cotton seed meal, residues from a chicken slaughter house, peanuts, fodder yeast, cheese whey and corn steep liquor [7,13,15]. Use of sewage sludge as a sole raw material for the production of biopesticide and other value-added products (biofertilisers, bioplastics, enzymes and others) will not only reduce substantially the overall cost of production, but will also minimise a longstanding environmental problem of sludge disposal. Experiments using shake flasks revealed that sewage sludge supports Bt-based biopesticide production without supplementation of nutrients from external source. The spore concentration and toxin synthesis in sludge medium were as high [14] as in commercial media [6]. Foam formation was a serious problem in sludge fermentation.

The antifoam agents used in current practice (block copolymers like PPG and silicone-based chemical antifoam agents) exert a negative effect on cell and product yield. The yield of endotoxin was lowered by 22% with slight inhibition of bacterial growth when polymeric antifoam propynol was used to control the foam [20]. These authors also found that a mixture of soap stock and silicone emulsion (as an antifoam agent) did not change the yields of cells and endotoxin. Similar contentions are also observed in our work using sludge as a Bt production medium where entomotoxicity decreased with PPG concentration and remained almost constant with the use of "Antifoam A".

In the production process using Bt, the entomotoxicity increased with an increase in total number of spores. However, the entomotoxicity per spore decreased at increased spore concentration [14]. Similar observations were also made in this work (Table 3). PPG lowered the cell concentration, spore concentration and percent sporulation. Thus, relative (or overall) entomotoxicity decreased in spite of increased entomotoxicity per spore (Table 3).

The control of process parameters required more attention as we increased the scale of operation from shake flasks to the laboratory fermentor. In order to achieve similar mass transfer conditions (or desired DO concentration) in the fermentor, more intensive conditions of agitation and aeration are required, which frequently give rise to pronounced foam formation. The sludge medium contains proteins and bacterial cell debris that enhance foam formation in the fermentation broth, as proteins are well known for their foaming properties [20]. The foam creates an additional non-homogeneity in the liquid due to floatation of physiologically different microorganisms. This is a serious problem in application of sludge as a raw material for commercial Bt production.

The natural oils could be used as antifoam agents in order to reduce many of these problems related to the use of synthetic antifoam agents. The natural oils, apart from controlling the foam, also help to enhance the DO concentration in the fermentation liquid. These oils act as oxygen vectors [12]. Oils added to the growth medium form an immiscible phase (mixture of oil and medium) in which oxygen has a higher solubility. This increases the DO concentration in the medium without necessitating an increased energy supply due to higher agitation and aeration [11].

Immediately after fast destruction of the oxygen-rich foam phase, an instant increase of DO concentration in the fermentor medium (sludge as well as TSYB) was observed (results not presented). This is because destruction of foam leads to increased contact (or renewal of contact) between the liquid phase and gas bubbles and that, in turn, results in an increase in DO. This implies that enforced destruction of foam to ensure a constant renewal of the interface contact between liquid and air bubbles is necessary to

increase DO. This is partially accomplished by an intensive mixing either mechanically or through turbulence or by liquid circulation. Thus, the use of an antifoam agent is essential not only to control the foam but also to renew the gas–liquid contact for better transfer of oxygen. However, excessive addition of an antifoam agent may cause decreased growth and endotoxin yield as observed in this study (Figures 2 and 3) and by others [20]. Apart from decreasing the cell and product yield, the addition of excessive antifoam agent may lead to lowering of the DO concentration and formation of dense foam that could be difficult to destroy [16].

Foam formation depends on the concentration of sludge solid and on colloidal particles suspended in the sludge. The quantity of antifoam agent required to destroy foam increases with the sludge solids concentration. Increased sludge solids reflect an increased concentration of proteins and hence, more intense foaming. The requirement of antifoam agents also differs significantly with sludge type (primary, secondary, mixed) and sludge composition, which is expected to vary with time, season, plant location, type of wastewater treated, sludge treatment process and sludge thickening (if applied). Qualitatively, we observed very intense foaming in Bt fermentation of the mixed sludge than in secondary sludge from CUQ wastewater treatment plant. Foaming in thickened sludge (diluted to 25 g/l suspended solids) was much higher than in mixed sludge.

Conclusions

The following conclusions can be drawn: (a) PPG used for foam control in Bt fermentation is toxic for growth and decreases the yield of entomotoxicity; (b) Antifoam A was effective in foam control with less severe adverse effects; (c) natural oils can be used for effective foam control without affecting growth and toxin production; (d) canola, olive and peanut oils were very effective in foam control; (e) use of natural oils as antifoam agents enhanced the yield of toxin and spore production; (f) the oils containing higher amounts of monounsaturated fats enhanced the toxin yield; and (g) peanut and soybean oils were 20 times more efficient economically than PPG in foam suppression.

Acknowledgements

The authors thank the Natural Science and Engineering Research Council of Canada (grants A 4984, STR 202047 and SCF192190-96) for financial support.

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