Techno-economic Analysis of Bacillus thuringiensis Biopesticides Production from Wastewater and Wastewater Sludge


* Institut National de la Recherche Scientifique (INRS - ETE), 490, de la Couronne, Québec, Canada G1K 9A9
(E-mails: satinderbrar2003@yahoo.ca; brarsa@ete.inrs.ca; mausamverma@yahoo.com; tyagi@ete.inrs.ca)
**US Environmental Protection Agency, P.O. Box 17-2141, Kansas City, USA, KS 66117

Abstract: Detailed techno-economic analysis of alternative growth substrates, namely, raw wastewater sludge; hydrolyzed wastewater sludge; starch industry wastewater for Bacillus thuringiensis var. kurstaki HD-1 (Bt) biopesticides production in comparison with semi-synthetic commercial soyameal medium was carried out. The analysis was based on experimental results obtained using different process configurations (principally, batch type) considered as process scenarios. The estimated product costs (calculated via Excel program) were an outcome of net entomotoxicity (BIU/L) of formulations. The hydrolyzed wastewater sludge gave the lowest product cost of $Can 0.228/BIU with molasses as a phagostimulant. Likewise, Tween-80 amended fermentation of raw wastewater sludge yielded a competitive product cost of $Can 0.256/BIU. The discounted cash flow return rate (profitability) for a payback period of 10 years was highest for soyameal medium (79.1%); however, the profitability for alternative raw materials was also higher than 40% justifying the process economics. The study demonstrated that, within the range of parameters experimented and studied, the biopesticides production from wastewater and wastewater sludge was economically viable making them promising commercial options.

Keywords: Bacillus thuringiensis; soyameal; techno-economics; wastewater; wastewater sludge

INTRODUCTION

Wastewater and wastewater sludge has been extensively explored for production of Bt based biopesticides as a novel value-addition approach. In this context, various studies have been already carried out on isolation and identification of new Bt strains; process optimization; enhancement of entomotoxicity through pre-treatment and testing at laboratory and pilot scale fermenters; rheology and its effects on fermentation, downstream processing and formulation development under the umbrella of Bt-INRS process (Lachhab et al., 2001; Tirado-Montiel et al., 2003; Vidyarthi et al., 2002; Brar et al., 2004, 2005, 2006a,b; Yezza et al., 2005 a,b,c, 2006a,b; Barnabé et al., 2005; Mohammadi et al., 2006). However, to evolve and establish Bt-INRS process as an integrated technology, marketing needs to be explored. The marketability and extensive use of Bt based biopesticides is a function of production as well as formulation costs (Lisansky, 1993; Burges, 1998). Earlier techno-economic analysis carried out on soyameal-corn steep liquor medium established 50% of the cost incurred by formulation ingredients (Rowe and Margaritis, 2004). However, this study stand alone cannot predict the economical analysis of other raw material based biopesticides production and moreover encompasses following drawbacks:

1. The entomotoxicity of the broth was taken to be very low at 0.61, 1.21 and 1.73 BIU/L for batch, low density and high density fed-batch fermentation, respectively.
2. The operation period assumption was typically higher at 330 d rather than the usually recommended value (Peters and Timmerhaus, 1980).
3. The fermenter size was large which could have been otherwise divided into multiple fermenters.
4. Discounted cash flow rate of return (DCFRR), an important parameter which addresses the risk encountered by a typical process technology, was not calculated.
5. The sole harvesting process was assumed to be centrifugation.
6. The input data required for costing was drawn from old publications based on results with higher spore and biomass concentration as indicators of higher entomotoxicity. In fact, spore concentrations cannot give the true picture of entomotoxicity of the fermentation broths. The entomotoxicity was compared to 16000 IU/mg standard formulation which has become obsolete in the current scenario. The Bt biopesticides production has undergone tremendous change in terms of search for alternative economical raw materials, optimization of process parameters, higher performance strains and entomotoxicity measurements. Hence, some of the interpretations may be over or underestimated.

In the present ordeal to produce economical biopesticides, wastewater and wastewater sludge were used as raw materials which warrants extensive study to predict the economical repercussions of the alternative technology (“Bt-INRS process”). Thus, this leads us to the main questions addressed in this article:

- Can the wastewater and wastewater based biopesticide technology become a competitive alternative to conventional soya based technology and what kind of developments and future research is necessary to make it more cost-effective?
- Can the wastewater and wastewater based biopesticide technology provide an economically viable opportunity for waste management, suitable for future scale-up and development of biopesticides thereof?

Hence, a detailed techno-economic analysis of Bt-INRS process is presented herein with possible process scenarios; projected cost simulations and break-even curves entailing future ramifications of the alternative technology based on results derived from fermentation at 10 and 150 L fermenter scale.

MATERIALS AND METHODS
All prices stated are in Canadian dollars ($Can), taken as equivalent to $ 1.12 (dated 18 May, 2006) for purposes of conversion, unless noted.

General Process Details
Process analysis and economic evaluation of the production of Bt biopesticides for different process scenarios was performed using the EXCEL program. The baseline biopesticides production plant capacity was assumed to be $10^7$ BIU/year based on stand-alone plant in southern Ontario, Canada (Rowe and Margaritis, 2004). The batch and fed-batch fermentation along with dry and liquid formulations were explicitly modeled along with modeling of recovery by centrifugation and ultrafiltration. The principal substrates used for Bt fermentation were – non-hydrolyzed sludge (NH), hydrolyzed sludge (TH), starch industry wastewater (SIW) and soyameal (conventional semi-synthetic medium) along with different process scenarios as provided in Table 1.

A generic flow sheet showing major unit operations for the Bt-INRS process is also illustrated in Figure 1. Although several items of equipment are not included in the flow sheet, their cost was taken into account in the economic analysis (as discussed later).

Upstream processing steps. Transportation of the substrates viz. wastewater sludge (WWS) and/or SIW was assumed to be carried out from the wastewater treatment/industrial site to the plant site at a fixed distance of 25 km.
Table 1. Different scenarios for equipment design and cost-estimation of Bt-INRS process

<table>
<thead>
<tr>
<th>Scenario (s) description</th>
<th>Code</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid formulation/suspension (I)</td>
<td>LS</td>
<td>Aqueous formulation development comprising harvesting of fermented broth by centrifugation.</td>
</tr>
<tr>
<td>Liquid formulation with recovery step of ultrafiltration (II)</td>
<td>UFLS†</td>
<td>Aqueous formulation development comprising harvesting of fermented broth by centrifugation and ultrafiltration.</td>
</tr>
<tr>
<td>Dry formulation with recovery step of ultrafiltration (III)</td>
<td>UFDF†</td>
<td>Dry formulation development comprising harvesting of fermented broth by centrifugation and ultrafiltration.</td>
</tr>
<tr>
<td>Dry formulation without ultrafiltration (IV)</td>
<td>DF</td>
<td>Dry formulation development comprising harvesting of fermented broth by centrifugation.</td>
</tr>
<tr>
<td>Molasses as phagostimulant in molasses formulation (V)</td>
<td></td>
<td>Aqueous formulation development comprising harvesting of fermented broth by centrifugation and supplementation of molasses (0.2 % w/v) at formulation step.</td>
</tr>
<tr>
<td>Fed-batch process (NH sludge) fed-batch (VI)</td>
<td></td>
<td>Fed-batch strategy with remaining steps similar to LS scenario.</td>
</tr>
<tr>
<td>Tween fortification during Tween-80 fermentation (NH sludge) (VII)</td>
<td></td>
<td>Tween-80 addition during fermentation with remaining steps similar to LS scenario.</td>
</tr>
</tbody>
</table>

Subsequently, the substrates were pumped by using a centrifugal pump ($P_{c1}$) into the storage tank (T-1) which was made up of stainless steel as the substrates are normally at acidic pH (pH=3-3.5 and 5-6.5 for SIW and WWS, respectively). There were numerous valves (number as $V_n$, $n=1,2,...$etc.) in the process diagram to control flow of various streams.

The solids preparation steps were carried out in a systematic manner as presented in our earlier studies (Lacchab et al., 2001; Brar et al., 2004, 2005, 2006a,b). The fermentation was considered to be carried out for a total period of 36-38 h for a batch scenario (Vidyarthi et al., 2002; Brar et al., 2005) and 48 h for fed-batch fermentation (Yezza et al., 2005 a).

**Downstream processing**

After the completion of fermentation, the pH of the fermented broth was lowered in-situ from $7\pm0.1$ to $4\pm0.1$ with 6M H$_2$SO$_4$ (50 ml of H$_2$SO$_4$ was approximately used per 10 L of the fermented broth) inside the fermentor (Brar et al., 2006a). The Bt fermented broths (NH/TH/SIW/soya) were harvested by the use of disc-vane centrifuge (DVC-2) and the supernatant was concentrated using ultrafiltration (Brar et al., 2006a; Adjalle et al., 2006). Different optimized adjuvants for each fermented broth were mixed along with the centrifugate in the mixing tank for liquid formulations (Brar et al., 2004, 2005, 2006a).

**Economic Analysis**

Economic analysis was carried out according to the standard economic protocols as given in Ulrich (1984) and Peters and Timmerhaus (1980), unless stated otherwise. The plant was assumed to operate 24 h/d, 300 days per year, resulting in 126 batches (for all scenarios) and 107 (for fed-batch) per annum with fermentation cycle of 56-58 h and 67 h for batch and fed-batch process, respectively. The capital cost of different equipments and profitability analyses was based on production of different formulations. The calculations for process equipment design, number of identical units, installed cost, total capital investment, operating costs, revenue and overall profitability (DCFRR – discounted cash flow rate of return) were carried out in the EXCEL program. The total capital cost for each of the process scenarios is given in Table 2.
Figure 1. Flow diagram of “Bt-INRS” production process (includes all possibilities, namely, scenario with centrifugation and ultrafiltration as well as dry and liquid formulations). Acid, alkali and anti-foam addition tanks are not shown for the simplicity of the schematic, but they have been included in the equipment design and costs.

BM- Ball mill; Con–Conveyor; CT-Cooling tower; DM–Demineralization; DVC-Disc vane centrifuge; F–Fermenter; GFB-Gas fuelled boiler; GP-Gear pump; IM-Impeller motor; MeT-Media tank; MT-Mixing tank; PC-Centrifugal pump; PF-Pre-fermenter; PPF-Pre-pre-fermenter; SD-Spray dryer; T-Storage tank; UF–Ultrafiltration; V–Valves.
RESULTS AND DISCUSSION

Total Capital Cost

Table 2 presents total capital investment for each of the substrates with various process scenarios. For a stand-alone plant manufacturing liquid formulations, total capital cost was approximately $Can 21 million for NH-Tween -80; $Can 18 million for TH-Molasses; $Can 20 million for SIW-Molasses and $Can 20 million for soya-Molasses, the best scenarios of each of the substrates.

Production Cost Analysis

Raw material costs for different process scenarios are presented in Table 2. It was found that TH-UFDF scenario showed the least raw material costs. Sludge transportation costs as given by Boileau & Associates (1989) comes out to be $Can 58 per ton at 3 tons per day over a distance of 25 km for a thickened sludge at 22% solids incorporating the costs of diesel and natural gas prevalent currently into a model developed at INRS (Modèle Métix).

Comparison of Techno-Feasibility of Different Process Scenarios

When the techno-feasibility of different batch (until, specified otherwise) fermentation scenarios, namely, four different substrates, NH sludge, TH sludge, SIW and soymeal — scenario-I: LS; scenario-II: UFLS; scenario-III: UFDF; scenario-IV: DF; scenario-V: Molasses; scenario-VI: Fed-batch and scenario-VII: Tween-80 was considered, it was found that the use of molasses as a phagostimulant, sticker and UV screen was found to be the best case for all scenarios and more particularly, NH sludge showed the best scenario with the use of Tween-80 as a surfactant. It was found that TH sludge came out to be the best raw material for Bt fermentation despite the inclusion of hydrolysis process, which incurred additional equipment costs. However, utilization of fermenter as a hydrolyzer minimizes the cost of equipment and hence, production costs.

It was observed that at the base production scale of 3 x 10^7 BIU/year, the direct production costs for SIW was the lowest at $Can 0.064/BIU (Table 2) which may be due to the lower need of acid and base for pH adjustment as well as lower centrifugation costs (as centrifugation was not required at the preliminary stage of medium amendment). Meanwhile, the DCFRR results showed that soymeal medium gave the highest DCFRR of 79.1% in a payback period of 10 years which could be due to the reduction in fixed charges (no need of centrifugation in the upstream processing and lower steam requirements as sterilization time before fermentation would be 15 min compared to 30 min for other alternative materials as well as lower quantity of adjuvant in formulations). The DCFRR (%) followed the order: Soya (79.1) > TH sludge (63.27) > NH sludge (61.1) ≥ SIW (60.03).

However, despite the higher profitability for soymeal medium, the TH sludge comes out to be a winner with lower product price of $Can 0.228/BIU when compared to other raw materials for Bt fermentation. In terms of the product entomotoxicity, 66B product was obtained for TH sludge and on the contrary 69B was obtained for NH sludge, this is far from the actual commercial product, yet much higher when compared to the control scenario of soymeal medium (54B, highest possible net Tx) as a semi-synthetic conventional medium. Meanwhile, when different raw materials were compared amongst each other, under the best-estimate sale price and moderate production scale assumptions, much appears to be gained by employing batch fermentation of hydrolyzed wastewater sludge (TH sludge scenario) as opposed to other raw materials.

Conversely, there is little incentive to employ ultrafiltration to recover the lost Tx in the supernatant during centrifugation so as to push the Tx towards higher values. In fact, the equipment cost needs to be seriously considered (perhaps a cost-benefit analysis could be done) before adopting this option for a full-scale plant. Moreover, the fed-batch process seems to push the net Tx towards higher value as well as reduce the product cost as seen in NH sludge (studies in our laboratory have been only carried out on this alternative). Thus, fed-batch needs to be explored as a sustainable and Tx boosting option for SIW and TH sludge too. However, the capital and product cost calculations developed in this study can be extended to the techno-economics of other fermentation technologies to develop value-added products from wastewater/wastewater sludge.
Table 2. Total costs and DCFRR of different substrates for Bt-INRS process

| Scenario (s) | Raw material cost ($Can/year)$ | Total capital investment ($Can 000) | Annual total product cost ($Can/year) | Net Tx (BIU/L)$ | Total product cost, $Can/BIU$***
<table>
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<tbody>
<tr>
<td>NH-LS</td>
<td>1783438</td>
<td>21492</td>
<td>8232536</td>
<td>2.88 (55B)</td>
<td>0.274</td>
</tr>
<tr>
<td>NH-UFLS</td>
<td>1610626</td>
<td>26837</td>
<td>9352735</td>
<td>3.04 (58B)</td>
<td>0.311</td>
</tr>
<tr>
<td>NH-UFDFT</td>
<td>179685</td>
<td>36416</td>
<td>9822422</td>
<td>3.04 (58B)</td>
<td>0.327</td>
</tr>
<tr>
<td>NH-DF</td>
<td>189667</td>
<td>31075</td>
<td>8608736</td>
<td>2.88 (55B)</td>
<td>0.287</td>
</tr>
<tr>
<td>NH-Fed-batch</td>
<td>1890764</td>
<td>22755</td>
<td>8774800</td>
<td>3.044 (58.2B)</td>
<td>0.292</td>
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<tr>
<td>NH-molasses</td>
<td>1535725</td>
<td>21169</td>
<td>7954211</td>
<td>3.221 (61B)</td>
<td>0.265</td>
</tr>
<tr>
<td>NH-Tween-80</td>
<td>1392594</td>
<td>20846</td>
<td>7700526</td>
<td>3.646 (69B)</td>
<td>0.256</td>
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<tr>
<td>TH-LS</td>
<td>1652138</td>
<td>18427</td>
<td>7221960</td>
<td>5.848 (55B)</td>
<td>0.241</td>
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<tr>
<td>TH-UFLS</td>
<td>1560856</td>
<td>23817</td>
<td>8355069</td>
<td>6.19 (66B)</td>
<td>0.279</td>
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<tr>
<td>TH-UFDFT</td>
<td>155345</td>
<td>33411</td>
<td>8854661</td>
<td>6.190 (58.5B)</td>
<td>0.296</td>
</tr>
<tr>
<td>TH-DF</td>
<td>164430</td>
<td>28021</td>
<td>7624888</td>
<td>5.848 (55B)</td>
<td>0.254</td>
</tr>
<tr>
<td>TH-molasses</td>
<td>1468062</td>
<td>17861</td>
<td>6868652</td>
<td>6.608 (62.4B)</td>
<td>0.228</td>
</tr>
<tr>
<td>SIW-LS</td>
<td>1307780</td>
<td>20977</td>
<td>7714862</td>
<td>2.44 (55B)</td>
<td>0.258</td>
</tr>
<tr>
<td>SIW-UFLS</td>
<td>1227301</td>
<td>26153</td>
<td>8804954</td>
<td>2.6 (58B)</td>
<td>0.293</td>
</tr>
<tr>
<td>SIW-UFDFT</td>
<td>205270</td>
<td>35747</td>
<td>9755519</td>
<td>2.6 (58B)</td>
<td>0.325</td>
</tr>
<tr>
<td>SIW-DF</td>
<td>218730</td>
<td>30571</td>
<td>8586613</td>
<td>2.44 (55B)</td>
<td>0.287</td>
</tr>
<tr>
<td>SIW-molasses</td>
<td>1164291</td>
<td>20479</td>
<td>7411759</td>
<td>2.757 (61.3B)</td>
<td>0.248</td>
</tr>
</tbody>
</table>

Soya-LS: 2476355 21799 10455028 2.772 (48B) 0.357 81.53
Soya-UFLS: 2342818 26749 11437821 2.93 (50.3B) 0.389 71.97
Soya-UFDFT: 1152483 36317 12190461 2.93 (50.3B) 0.415 57.75
Soya-DF: 1152484 30859 10922146 2.93 (50.3B) 0.372 60.81
Soya-molasses: 2199510 20799 9799766 3.132 (54B) 0.335 79.07

$Net Tx (BIU/L) = \frac{T_{\text{translation}} \times V_{\text{translation}}}{L}$ of medium; respective values in parentheses represent the final Tx of the formulation reported as BIU/ U.S. gallon represented as “B”.

**Total raw material cost = raw materials for fermentation and formulation

CONCLUSIONS

The entomotoxicity after fermentation, consequent recovery during the harvesting step (centrifugation and ultrafiltration) and adjuvant addition leading to formulation stability and increase in net Tx after formulations were the three most important factors controlling the Bt production at an industrial scale. The major cost regulating components of the Bt production process is additional link-up of the equipments like ultrafiltration vis-à-vis the recovery of entomotoxicity. Meanwhile, the air compressor and the fermenter single handedly govern the final production cost of the product by affecting both the equipment cost and the operational expenses. The hydrolyzed sludge gave the lowest product cost of $Can 0.228/BIU. The use of molasses as a multi-adjuvant, for phagostimulation, sticking and UV resistance lowered the total product cost by approximately 25% for all substrates. The addition of Tween-80 in the non-hydrolyzed sludge as a surfactant modified the sludge rheology and lowered the product cost to $Can 0.256/BIU when compared to other BT fermentation substrates. The discounted cash flow return rate (measure of profitability) was highest for soya meal medium (79.1%); however, the profitability was also within the economical range for the alternative raw materials.

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