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Review Paper

Application of Membrane Separation Technology in Downstream Processing of *Bacillus thuringiensis* Biopesticide: A Review

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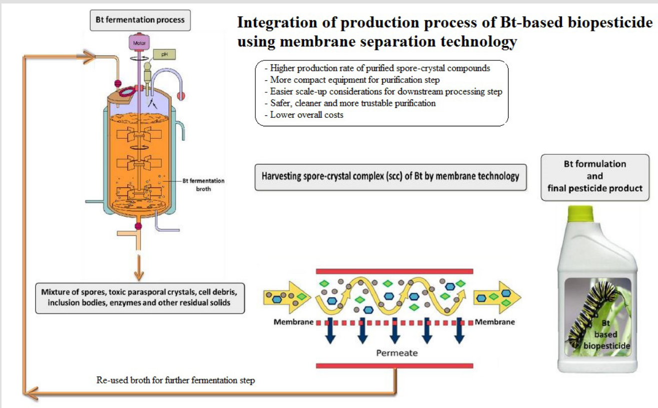
GRAPHICAL ABSTRACT



HIGHLIGHTS

- Bt has been extensively used in biopesticides due to its safe records
- Separation/purification of Bt is a crucial link between production and application
- Bt purification dictates economy, longer shelf life and enhanced field efficacy
- Membrane technology has been recently introduced to downstream processing of the Bt

ABSTRACT

Bacillus thuringiensis (Bt) has been extensively used in biopesticidal formulations due to its safe environmental and human health records. The widespread use of Bt is often challenged by production as well as formulation costs which are in direct contact with downstream processing, i.e. the separation/purification step. Downstream separation/purification efficacy governs the marketability of a product by affecting potency and aiding in further processing during formulation development. Separation/purification of Bt from fermentation broth is a crucial link between production and application and dictates economy, longer shelf life after formulation, ease of application and enhanced field efficacy. There are various methods like chemical precipitation, centrifugation and etc. which impede the efficacy of Bt recovery; however, all of them have their own limitations and drawbacks. In this regard, membrane separation technology has been recently introduced for downstream processing of the Bt biopesticide. This article comprehensively reviews recent advances in downstream processing of Bt based biopesticides incorporating the effect of different membranes and membrane processes.

1. Introduction

Population growth has always been and will remain one of the main drivers of the energy, water and food demand, along with economic and social developments [1, 2]. As the world population rises dramatically, we

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will also be faced with a more serious environmental crisis like air pollution, deforestation and water pollution which could lead to serious health problems in all parts of the world. Figure 1 illustrates the most important global crisis and their relation to population growth, comprehensively. By some projections there will be almost 9 billion people inhabiting the planet by 2050 and one of the many issues raised by this expected population surge (more than 35%) is how to feed all those people [3]. In other words, with a 35% increase in the global population, crop production will need to double to feed that population, and the most important question is "where will we find enough food for 9 billion?"

Nowadays modern farming offers solutions, e.g. fertilizers [4], herbicides [5], and pesticides [6] that are commonly used to reduce crop losses due to pests both before and after harvest, and get a much more cleaner crop with bigger returns per acre, and more varieties or cultivars of a certain species of plant are made available to farmers to grow for better yield, less lodging, more robustness and durability throughout the growing stages, and more growth or competitiveness over other plants that would be considered weeds. For instance, Figure 2 shows the amount of pesticides that were applied on arable land of the world during 2005-2009. It is to be noted that without such important crop protection and pest control technologies, food production would decline, many fruits and vegetables would be in short supply, and consequently the price of food would rise. It is worth quoting that among the above mentioned solutions, the *Pesticide* technology is now highlighted.

Pesticides allow consumers to purchase high quality products that are free of insect blemishes and insect contamination. Pesticides have been used to control organisms that are considered to be harmful and can be classified by target organisms (e.g., herbicides, insecticides, fungicides, rodenticides and pediculicides), as is shown in Figure 3, and also by chemical structure (e.g., organic, inorganic, synthetic or biological (biopesticide)). Within the last few decades, the use of synthetic and chemical pesticides raises a number of environmental and health problems [7,8]. Many studies have examined the effects of chemical pesticides and indicated that their exposure is associated with long-term health problems such as respiratory problems, memory disorders, depression [9], and different cancers like leukemia, lymphoma, brain, kidney, breast, prostate, pancreas, liver, lung and skin cancers [10]. Table 1 presents an overview of health effects of about 30 conventional chemical pesticides.

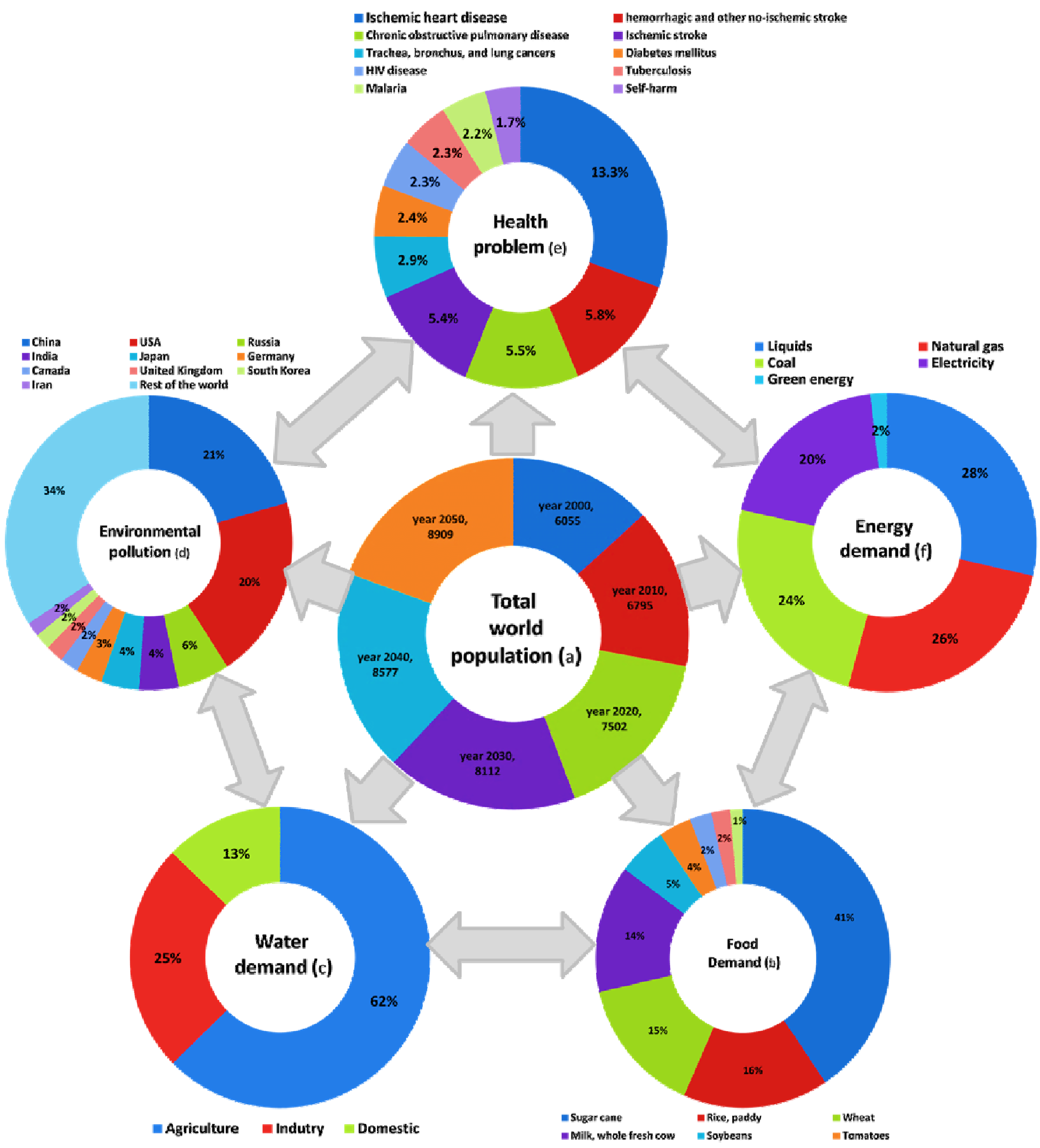

Fig. 1. The relationship between world population growth and global crisis, e.g. energy demand, water demand, food demand, environmental pollution and health problems.(a: World population increase trend and its distribution since 2000 to 2050 (millions); b: List of world most valuable crops production (MT, in 2012); c: Water consumption in the world during the 20th Century, in km3/y; d: Comparison of global energy consumption pattern for industries in 2030; e: Global Burden of Disease Study 2010 (GBD 2010) Results by Cause 1990-2010; f: World carbon dioxide emissions from the consumption and flaring of fossil fuels (2006, million metric tons of Carbon dioxide)).

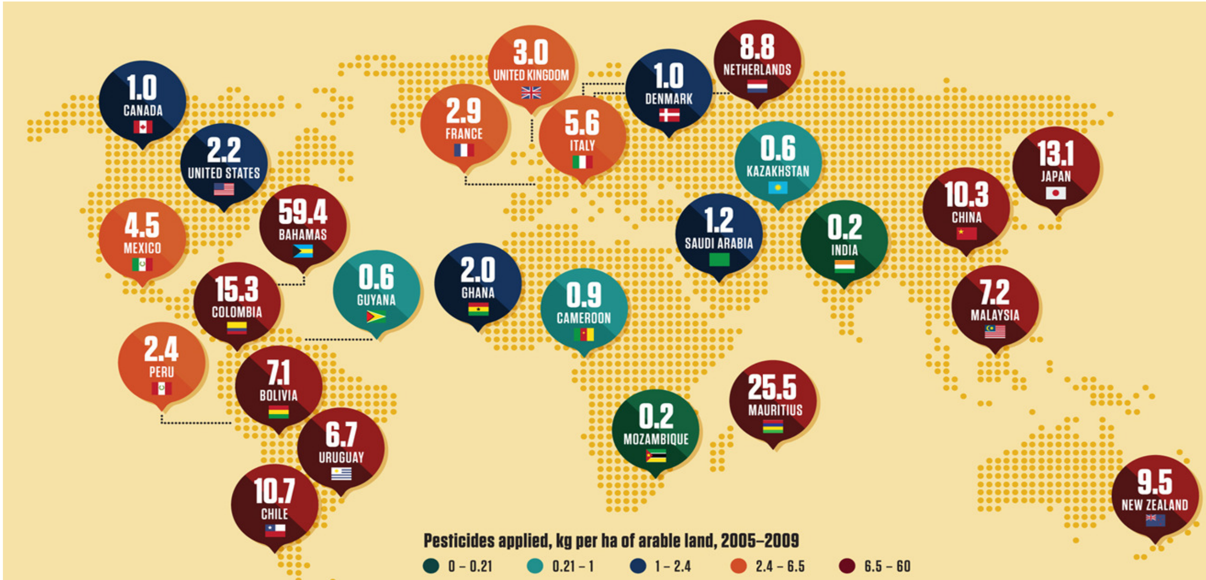

Fig. 2. Amount of pesticides were applied on arable land of the world during 2005-2009 (kg/ha) [11].

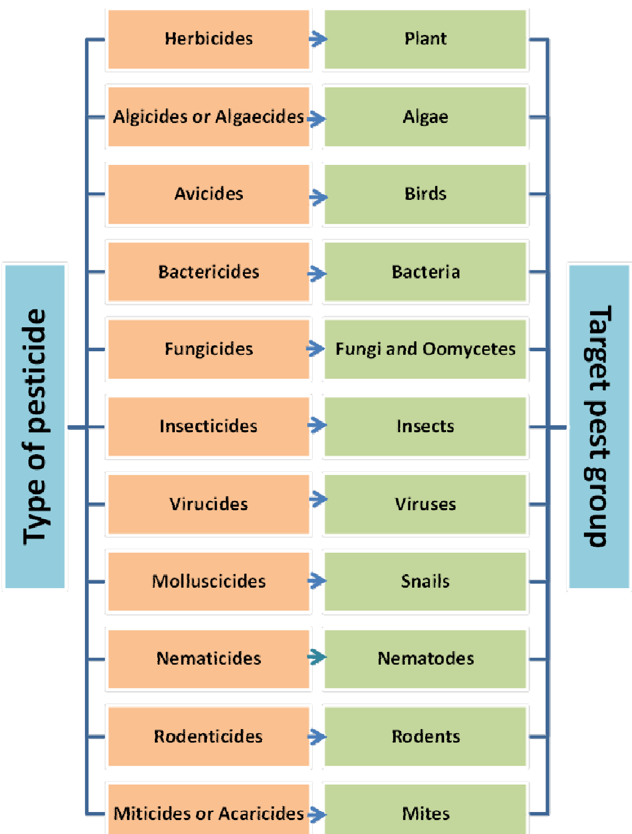

Fig. 3. Classification of chemical pesticides based on the target pest.

Despite many years of effective control by conventional agrochemical insecticides, a number of factors are threatening the effectiveness and have continued the use of these agents. These include the development of insecticide resistance and use-cancellation or de-registration of some insecticides due to human health and environmental concerns. Therefore, an eco-friendly alternative is the need of the hour. Improvement in pest control strategies represents one method to generate higher quality and greater quantity of agricultural products. Therefore, there is a need to develop biopesticides which are effective, biodegradable and do not leave any harmful effect on the environment [12].

In this work, we reviewed recent advances in downstream processing of Bt (*Bacillus thuringiensis*) based biopesticide, comprehensively. Various aspects of membranes application for the separation of spore and crystal from Bt fermentation broth were investigated. In other words, this study can provide critical and practical data for those interested in developing Bt biopesticide based on its downstream processing which can lead to a better end-product formulation.

2. Biopesticide

Biopesticides, also known as biochemical pesticides, are naturally occurring substances that control pests by nontoxic mechanisms [13-15]. They pose less threat to the environment and to human health, are inherently less harmful, designed to affect only one specific pest or, in some cases, a few target organisms, are often effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoid pollution problems. In general, biopesticides fall into three major categories: plant-incorporated protectants (PIPs), biochemical, and microbial pesticides [16]. Table 2 shows an overview of microbial biopesticides based on bacteria, fungi and viruses which are registered and available on the market in some countries, with respect to their target organisms.

Globally, there are 175 registered biopesticide active-ingredients and 700 products available in the market. The global market for biopesticides was valued at US $1.3 billion in 2011, and it is expected to reach US $3.2 billion by 2017. The increasing demand for residue-free crop production is one of the key drivers of the biopesticide market. The growing organic food market and easier registration than chemical pesticides are other important driving factors for the growing biopesticide market [17,18].

2.1. *Bacillus thuringiensis* (Bt)

The most successful insect pathogen used for insect control is the bacterium *Bacillus thuringiensis* (Bt), which is a gram-positive, rod-shaped, aerobic, and sporulating bacterium closely related to the omnipresent soil bacteria *Bacillus cereus* [19]. It can remain latent in the environment even in adverse conditions for its development. Bt can be found in soil, insects and their habitats, stored products, plants, forest and aquatic environments [20-22]. The species is distinguished from *B. cereus* by the presence of a parasporal inclusion body (crystal) of protein origin, formed during sporulation [23]. Crystal formation is conferred by genes carried on a plasmid. The genes, which encode Cry / Cyt proteins, become active during sporulation because they are controlled by a dedicated RNA polymerase that is also synthesized specifically while spores are forming [24-26].

Table 1
Health effects of 30 commonly used pesticides.

	Health Effects						
	Cancer	Endocrine Disruption	Reproductive Effects	Neurotoxicity	Kidney/Liver Damage	Sensitizer/ Irritant	Birth Defects
Herbicides							
2,4-D*	X	X	X	X	X	X	X
Benfluralin					X	X	
Bensulide				X	X	X	
Clopyralid			X			X	X
Dachthal	Possible	X			X	X	
Dicamba*			X	X	X	X	X
Diquat Dibromide			X		X	X	
Fluazifop-p-butyl			X		X		X
Glyphosate*	X	X	X		X	X	
Isoxaben	X				X		
MCPA		X	X	X	X	X	
MCPP	Possible	X	X	X	X	X	X
Pelargonic Acid*						X	
Pendimethalin*	Possible	X	X			X	
Pronamide	Probable	X			X	X	
Triclopyr			X		X	X	X
Trifluralin*	Possible	X	X		X	X	
Insecticides							
Acephate	Possible	X	X	X		X	
Bifenthrin*§	Possible	Suspected		X		X	X
Carbaryl*	X	X	X	X	X	X	X
Dichlorvos	X	Suspected		X	X	X	
Fipronil	Possible	X	X	X	X	X	
Imidacloprid			X		X		X
Malathion*	Possible	X	X	X	X	X	X
Permethrin*§	X	Suspected	X	X	X	X	
Trichlorfon	X	X	X	X	X		X
Fungicides							
Azoyystrobin					X	X	
Myclobutanil		Probable	X		X		
Sulfur						X	
Ziram	Suggestive	Suspected		X	X	X	
Totals:	17	18	19	14	24	25	11

* These pesticides are among the top 10 most heavily used pesticides in the home and garden sector from 2006-2007, according to the latest sales and usage data available from EPA (2011).
§ EPA lists all synthetic pyrethroids under the same category. While all synthetic pyrethriods have similar toxocological profiles, some may be more or less toxic in certain categories than others. See Beyond Pesticides' synthetic pyrethriod fact sheet at bit.ly/TLBuP8 for additional information. (Source: www.beyondpesticides.org)

Today, commercial activities have concentrated on two strategies by Bt to overcome plant disease challenges, namely the development of Bt transgenic crops, and the development of pesticide products based on Bt [27]. The United States has enthusiastically embraced the development of Bt agriculture. However, Bt crops are grown in 25 other countries and the number of countries adopting Bt crops and the amount of land set aside for their cultivation has shown a continued upward trend for 15 years (see Figure 4) [28]. Bt agriculture is expanding on every continent except Europe, which persists with its absurdly byzantine approach to all genetically engineered crops. Remarkably, the small African nation of Burkino Faso grows more Bt crops than all of Europe. The total global area devoted to Bt crops in 2009 was >50 million hectares (36% of all biotech crops), made up of 21.7 million hectares of Bt-only crops and 28.7 million hectares of crops with Bt stacked with herbicide tolerance [29,30]. Although Argentina and Brazil currently hold second and third place in the global rankings for Bt agriculture, China and India have seen the most rapid adoption [31].

The first commercial product of Bt was named ''Sporeine'' in France in 1938 [32]. However, many formulations have not delivered effectively in a field owing to various environmental factors like ultraviolet radiation, rain, pH, temperature and foliage physiology which impede efficacy of Bt formulations [33, 34].

Table 2
Microbial Biopesticides for the Control of Plant Pathogens, [35].

Species/strain	Type	Target
BACTERIA		
Bacillus popilliae	Insecticide	Popilla japonica
B. thuringiensis var. aizawai	Insecticide	Galleria melonella
B. thuringiensis	Insecticide	Dipteran larvae
B. thuringiensis var. kurstaki	Insecticide	Lepidopteran larvae
B. thuringiensis var. xentari	Insecticide	Lepidopteran larvae
B. thuringiensis var. San Diego	Insecticide	Coleopteran larvae
B. thuringiensis var. tenebrionis	Insecticide	Coleopteran larvae
B. thuringiensis EG2348	Insecticide	Lymantria dispar
B. thuringiensis EG2371	Insecticide	Lepidopteran larvae
B. thuringiensis EG2424	Insecticide	Coleopteran larvae
Burkholderia cepacia	fungicide	Soil-borne fungi, nematodes
Pseudomonas fluorescens	fungicide	Soil-borne fungi
P. syringae ESC-10, ESC-11	fungicide	Post-harvest fungi
P. chlororaphis	fungicide	Soil-borne fungi
P. aureofaciens Tx-1	fungicide	Antracnose, soil-borne
Bacillus subtilis	fungicide	Soil-borne fungi
B. subtilis FZB24	fungicide	Soil-borne
B. subtilis GB03	fungicide	Soil-borne and wilt
B. subtilis GB07	fungicide	Soil-borne fungi
Streptomyces griseoviridis K61	fungicide	Various fungi
S lydicus	fungicide	Soil-borne
Agrobacterium radiobacter K84, K1026	bactericide	Crown gall A. tumefaciens
Ralstonia solanacearum non-pathogenic	bactericide	Pathogenic R. solanacearum
Pseudomonas fluorescens A506	bactericide	Frost damage, fire blight (E. amylovora)
Bacillus firmus	nematicide	Nematodes
Pseudomonas syringae pv. tagetis	herbicide	Cirsium arvense
Xanthomonas campestris pv. poae	herbicide	Poa annua
FUNGI		
Species/strain	**Type**	**Target**
Beauveria bassiana	Insecticide	White fly
Verticillium lecanii	Insecticide	White fly
Paecilomyces fumosoroseus	Insecticide	White fly
Metarrhizium anisopliae	Insecticide	Black beetle
Lagenidium giganteum	Insecticide	Mosquitoes
Trichoderma polysporum, T. harzianum	fungicide	Soil-borne fungi
T. harzianum KRL-AG2	fungicide	Soil-borne fungi
T. harzianum	fungicide	Foliar fungi
T. harzianum, T. viride	fungicide	Various
T. viride	fungicide	Various
T. lignorum	fungicide	Vascular wilt
Trichoderma spp	fungicide	Soil-borne
Ampelomyces quisqualis M-10	fungicide	Powdered mildew
Talaromyces flavus V117b	fungicide	Soil-borne fungi
Gliocladium virens GL-21	fungicide	Soil-borne fungi
G. catenulatum	fungicide	Soil-borne fungi
Fusarium oxysporum non-pathogenic	fungicide	Pathogenic Fusarium
Pythium oligandrum	fungicide	Phytium ultimum
Phlebiopsis gigantean	fungicide	Heterobasidium
Coniothyrium minitans	fungicide	Sclerotinia sclerotiorum
Candida oleophila I-182	fungicide	Post-harvest rot
Myrothecium verrucaria	nematicide	Nematodes
Paecilomyces ilacinus	nematicide	Nematodes
Phytophthora palmivora MWV	herbicide	Morrenia odorata
Colletotrichum gloeosporioides	herbicide	Cuscuta and various
C. gloeosporioides f.sp. malvae	herbicide	Malva pulsilla
C. g. f.sp. aeschynomene	herbicide	Curty indigo
C. coccodes	herbicide	Abutilon theophrasti
C. truncatum	herbicide	Sesbania exalta
Alternaria cassia	herbicide	Senna obtusifolia
VIRUSES		
Species/strain	**Type**	**Target**
Granulosis virus	Insecticide	Byctiscus betulae, codling moth
Pine sawfly NPV	Insecticide	Diprion similis
Heliothis NPV	Insecticide	Helicoverpa zeae
Gypsy moth NPV	Insecticide	Lymantria dispar
Tussok moth NPV	Insecticide	Orgyia pseudotsugata
Mamestria brassicae NPV	Insecticide	Heliothis
Spodoptera exigua virus	Insecticide	S. exigua
Bacteriophage of P. tolaasii	fungicide	Bacterial rot of mushroom

Formulation is a crucial link betdween production and application, and dictates economy, longer shelf life, ease of application and enhanced field efficacy. The characteristics and composition of biopesticidal formulations vary with a wide range of issues, e.g. type of habitat (foliage; soil; water; warehouse; size), pathogen (type; characteristics; regeneration mechanism and factors), rheology of technical material (viscosity; particle size; density), insect species (feeding habits; feeding niche; life cycle), mode of action (oral/contact); host pathogen environment interactions (behavioral changes; resistance; stability), mode of application (aerial; land) and application rate (L/ha and kg/ha). Formulations can also be classified into dry solid (dusts, granules, powders and briquettes) and liquid (termed ''suspensions''; oil or water based and emulsions) formulations.

Nowadays, many conventional formulations have been substituted by advanced versions like microencapsulations and microgranules to enhance residual entomotoxicity [6,36] and to overcome the adverse environmental effects. However, it is to be noted that the harvesting process, i.e. an earlier step before formulation, can govern the marketability of a product by affecting potency and aiding in further processing during formulation development. Hence, the main objective could be adoption of an integrated and proper approach for advances in downstream processing which can play an important role in Bt products development. In this light, the present review discusses advances in Bt harvesting technologies, in particular membrane separation technology, and tries to remark the most beneficial approach to have the best Bt pesticide product.

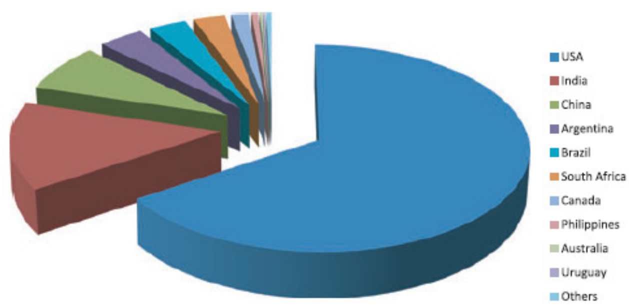

Fig. 4. Sharing out the Bt pie. More than 50 million hectares of Bt crops were grown commercially in 2008, the vast majority (>33 million hectares) in the USA. India, China, Argentina, Brazil, South Africa, Canada, Philippines, Australia and Uruguay were (in descending order by landarea) the other countries to grow >100 000 hectares. The minor growers (in descending order by land area) were Spain, Mexico, Colombia,Honduras, Burkino Faso, Czech Republic, Romania, Portugal, Germany, Poland, Slovakia and Egypt. The data include Bt-only crops and Bt stacked with other traits [37].

2.2. Bt harvesting techniques

The end product of fermentation of Bt broth is a mixture of spores, toxic parasporal crystals, cell debris, inclusion bodies, enzymes and other residual solids, which needs to be recovered efficiently to be utilized in a subsequent formulation step [38,39]. Since parasporal crystals are minute in size compared to bacterial cells, their efficient separation from fermented broth is a challenge. Harvesting microorganisms from submerged fermentation is often difficult due to the low concentration of products, their thermolabile nature and in some cases, poor stability. Key factors governing the choice of harvesting strategy include process throughput, physical characteristics of product and impurities and desired end-product concentration [40,41]. Moreover, depending on the desired entomotoxicity of the final product and scale of production, the processing required varies significantly. Therefore, it is very essential to identify an efficient method to harvest the spore crystal complex (SCC) of Bt in order to have maximum bioactivity and cost efficiency.

Various harvest processing methods such as the alginate immobilization method [42], acid or acetone precipitation, centrifugation [43,44], ultrafiltration (UF) [45], and microfiltration (MF) [46] have been reported earlier and the quality of the product obtained was variable depending upon the method used. Table 3 presents an overview of different methods applied for downstream processing of Bt biopesticide, i.e. recovery of spore and crystal from fermentation broth.

3. Membrane technology for *Bacillus thuringiensis* processing

Membranes and membrane processes have been used extensively for various applications including desalination [47,48], water/wastewater treatment [49-51], and specifically throughout the production, purification and formulation of biotechnology products [52-54]. The classification of various membrane processes according to their driving force and maturity is shown in Table 4.

Among various membranes and membrane processes, microfiltration (MF) (having a pore size between 0.1 µm and 5 µm) and ultrafiltration (UF) (having a pore size ranging from 10 nm to 0.1 µm) processes are well-known membrane-based separations in biotechnology and bioprocesses that are commonly used to recover proteins [55] (Cui, 2005) macromolecules and retain suspended colloids and particles [56,57]. These membranes are made from a variety of materials, including polymers (polyethersulfone (PES), polyethylene (PE), polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF), nylon, polyester, polycarbonate, cellulose acetate (CA), and regenerated cellulose), ceramics (aluminum and zirconium oxide), glasses (borosilicate glass fiber), and metals (silver and stainless steel). Regenerated cellulose, PES, and PVDF membranes are most commonly used for bioseparations due to their low protein binding characteristics [58,59].

Table 3
The advantages and disadvantages of the recovery methods commonly used for Bt-based biopesticides.

Methods	Materials and Equipment needed	Advantages/Disadvantages
Alginate immobilization	sodium alginate	✓ Minimum wastage, ✓ useful for small laboratories or small industries ✓ Initial investment is less ✓ Use supernatant
Acid or acetone precipitation	Acid or acetone	✓ Heavy wastage, ✓ Handling of corrosive chemicals, ✓ Toxicity and biomass yield is very less ✓ Low recovery efficiency ✓ Loss in supernatant
Centrifugation	Continuous centrifuge	✓ Moderate wastage, ✓ Toxicity and biomass yield is less compared to ultra-filtration method. ✓ Initial investment is high ✓ Recovery efficiency depends on the relative centrifugal force (RCF), and the RCF is difficult to increase for large amount of broth. ✓ Loss in supernatant
Ultrafiltration	Ultra-filtration unit with membrane cassette	✓ No wastage, ✓ Toxicity and biomass yield is high andcassette can be reused. ✓ Initial investment is less ✓ Use supernatant
Microfiltration	microfiltration unit with membrane cassette	✓ No wastage, ✓ High recovery efficiency ✓ Cost-intensive (frequent membrane replacement) ✓ Time-consuming ✓ Heavy membrane fouling ✓ Use supernatant

MF and UF processes are being integrated into both upstream and downstream bioprocessing applications [60]. Upstream applications include sterile filtration of fermentation media, pH control solutions, and gases (air, oxygen, and off-gases) [61-63]. Membranes with 0.1 µm pore size provide retention of both mycoplasma as well as larger organisms. Depth filtration, equipped by either MF or UF membranes, may also be used for turbid feed streams such as peptone solutions. Tangential flow microfiltration is used for medium exchange [64,65], perfusion and harvest [66]. Virus filtration may be used to protect cell cultures from the introduction of viral contaminants in media raw materials. Ultrafiltration and diafiltration have been used to remove glycine, hypoxanthine, and thymidine from the serum to provide selective pressure on serum dependent cell cultures [67,68]. Depth filtration may also be employed for product feed streams that are particularly difficult to filter with other types of membranes [69]. Virus filters are often used in the downstream processing of cell culture derived products to insure removal of both endogenous virus particles and any adventitious viruses that may enter into the cell culture through contaminated raw materials [70].Virus filtration was initially implemented as a tangential flow filtration operation but is now typically performed by normal flow filtration [71,72].

Table 4
Classification of membrane processes according to their driving forces and status.

Status of membrane processes

Category	Process	Maturity
Developed industrial membrane separation technologies	Microfiltration, ultrafiltration, reverse osmosis, electrodialysis	Well-established unit operations. No major breakthroughs seem imminent.
Developing industrial membrane separation technologies	Gas separation, pervaporation	A number of plants have been installed. Market size and number of applications served are expanding.
To-be-developed industrial membrane separation technologies	Carrier facilitated transport membranes, piezodialysis	Major problems remain to be solved before industrial systems will be installed on a large scale

Classification of membrane processes according to their driving forces

Pressure difference	Concentration (activity) difference	Temperature difference	Electrical potential difference
Microfiltration	Gas separation	Membrane distillation	Electrodialysis
Ultrafiltration	Pervaporation		
Nanofiltration	Carrier mediated transport		
Reverse osmosis	Dialysis		
Piezodialysis	Diffusion dialysis		

Pressure driven membrane processes

Status	Microfiltration	Ultrafiltration	Nanofiltration	Reverse osmosis	Piezodialysis
Membrane	(A)symmetric porous	Asymmetric porous	Composite	Asymmetric or composite	Mosaic membranes
Thickness	≈10–150 µm	≈ 150 µm	Sublayer ≈ 150 µm Top layer <1µm	Sublayer ≈ 150 µm Top layer <1 µm	≈ a few hundred µm
Pore size	≈0.05–10 µm	≈ 1–100 µm	≈ 2 nm	< 2 nm	Nonporous
Separation principle	Sieving mechanism	Sieving mechanism	sieving and electrostatic repulsion	Steric and electrostatic Repulsion	Ion transport
Membrane material	Polymeric, ceramic	Polymer, ceramic	polyamide	cellulose triacetate, aromatic polyamide, polyamide and poly (ether urea)	Cation/anion-exchange membrane

Concentration driven membrane processes

Status	Gas separation	Pervaporation	Carrier mediated transport	Dialysis	Diffusion dialysis
Membrane	Asymmetric or composite membranes with an elastomeric or glassy polymeric top layer	Composite membranes with an elastomeric or glassy polymeric top layer	Supported liquid membrane (SLM), emulsion liquid membrane (ELM), fixed carrier membranes, solvent swollen membrane	Homogenous	Ion exchange membranes
Thickness	≈0.1 to a few µm (for top layer)	≈0.1 to a few µm (for top layer)	20–150 µm (SLM), ≈ 0.1–1 µm (ELM)	10–100 µm	≈ a few hundred µm (100–500 µm)
Pore size	nonporous (or porous <1 µm)	nonporous	Nonporous		
Separation principle	Solution/diffuse on (nonporous membrane) Knudsen flow (porous membrane)	solution/diffusion	Affinity to carrier (carrier mediated transport)	Difference in diffusion rate, solution/diffusion	Donnan exclusion mechanism
Membrane material	Elastomeric and glassy polymer	Elastomeric and glassy polymer	Hydrophobic porous membrane as a support	Hydrophilic polymers	Anion/cation exchange membrane

Thermally driven membrane processes

Status	Membrane distillation
Membrane	Symmetric or asymmetric porous
Thickness	20–100 µm
Pore size	≈ 0.2–1.0 µm
Separation principle	Vapor-liquid equilibrium
Membrane material	Hydrophobic (polytetrafluoroethylene, polypropylene)

Electrically driven membrane processes

Status	Electrodialysis
Membrane	Cation-exchange and anion-exchange membranes
Thickness	≈ a few hundred µm (100–500 µm)
Pore size	Nonporous
Separation principle	Donnan exclusion mechanism
Membrane material	Hydrophobic (polytetrafluoroethylene, polypropylene)

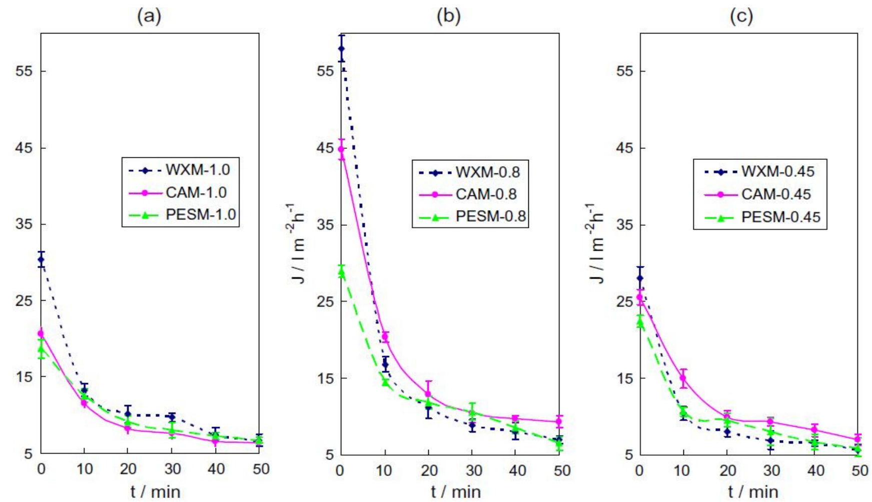

Fig. 5. Permeate flux variation of different pore size membrane for Bt recovery from fermented sludge in Chang et al. (2012) work [46].

3.1. Microfiltration experiments

Chang et al. [46] studied the performance of three types of MF membranes with various pore sizes for recovery of Bt from fermented sludge broth. In this work, cross-flow MF experiments using MF membranes made of WX-model cellulose, CA and PES (all hydrophilic) with pore sizes of 0.45, 0.8 and 1.0 µm were carried out. Figure 5 shows the permeate flux behavior of applied membranes when they were used for Bt recovery. The authors reported that in the first set of experiments, the cellulose acetate membrane gave the best performance in terms of both permeate flux and membrane restoration capacity.

Hence, the authors (see Ref. [46]) used this membrane for the next set of experiments. With a decrease in the membrane pore size up to 0.45 µm, the higher Bt recovery was achieved; however, a slight decline in flux could be observed. On the other hand, it can be seen (see Figure 5) that the obtained flux for the membrane with 0.8 µm is higher than that of the membrane with 1.0 µm. The authors concluded that this is due to higher possible pore fouling for larger pore size, which is in good agreement with the literature [73,74].

Moreover, the authors (see Ref. [46]) concluded that increasing the operating pressure (0.1 to 0.15 MPa) led to an increase in the permeate flux, but no further increases were observed for higher pressure than 0.15 MPa. The authors concluded that higher operating temperature also had a negative effect on the membrane fouling; however, further discussion on this issue (i.e. pore fouling) was not presented. Based on the obtained results, the permeate flux increased significantly as the velocity of the feed stream increased. In other words, less deposition on the membrane surface occurred as the velocity increased [46]. This is in good agreement with Marzban et al.'s work [75], which studied the optimization of spore/crystal recovery from Bt fermentation broth using the MF process.

Marzban et al. [75] investigated Taguchi optimization methodology for a sensitivity analysis study on effective operating parameters, e.g. feed pressure (2 to 40 psi), feed flow rate (80 to 160 LPH) and temperature (20 to 40 °C), for recovering spore/crystal (see Table 5). A polymeric membrane with 0.45 µm pore size, 115 µm thickness and 65% porosity was used for the experiments. Results indicated that the highest permeate flux (i.e. 51.85 kg/m^2.h) was achieved when pressure, flow rate and temperature of 40 psi, 160 PH and 30 °C, respectively, were used as operating conditions (see Table 5). Moreover, ANOVA analysis indicated that the most and the least effective parameters were the feed flow rate (Q) and feed temperature (T). These results were in good agreement with Chang et al.'s work [46]. The authors also concluded that the results of the Taguchi model under optimum conditions were validated with excess experiments, as is shown in Figure 6 [75].

In another work, Marzban et al. [76] studied the performance of two commercial polymeric membranes made of CA and PVDF and with the same pore size of 0.22 µm for recovery of spore-crystal from the Bt fermentation broth. In this work, the effect of various operating conditions on permeate flux and membrane fouling were investigated. Table 6 shows the measured permeate flux for PVDF and CA membranes under various operating environments. As could be observed, during most of the experiments, a higher permeate flux was achieved by use of the PVDF membrane. However, under 10 psi operating pressure, when feed flow rates of 400 and 120 liters per hour (LPH) were used, higher permeate flux was achieved for the CA membrane. In this work (see Ref. [76]), the authors also used scanning electron microscopy (SEM) to observe the scaling/fouling behavior of applied membranes (see Figure 7). The authors observed less surface scaling characteristics for the PVDF membrane. This is in good agreement with the literature [58,59]. Moreover, surface topography features of these membranes (i.e. PVDF and CA with 0.22 µm pore size) were characterized by atomic force microscopy (AFM). AFM analysis results indicated that the CA membrane has a rougher surface than that of the PVDF membrane (see Table 7). The authors concluded that a higher permeate flux for the PVDF membrane compared to CA was due to the less surface roughness and spore/crystal binding to the membrane surface [76].

Table 5
The experimental variables, their level and the obtained permeate flux based on Taguchi experimental design, in Marzban et al. work [75].

No.	P (psi)	Q (LPH)	T (°C)	Flux (kg/m^2.h)
1	20	80	20	42.08
2	20	120	30	40.42
3	20	160	40	50.30
4	30	80	30	40.42
5	30	120	40	46.09
6	30	160	20	49.06
7	40	80	40	39.76
8	40	120	20	46.22
9	40	160	30	51.85

Table 6
Permeate flux (max. and min.) for PVDF and CA membranes under various operating conditions [76].

Operation	Membrane			
	Flux for PVDF (kg.h^{-1}.m^{-2})		Flux for CA (kg.h^{-1}.m^{-2})	
	Max. ± SE[a]	*Min.*± SE	*Max.* ± SE	*Min.*± SE
P = 30 psi, **Q** = 40 LPH	61.44±1.56	22.50±0.86	49.44±1.96	21.49±0.76
P = 30 psi, **Q** = 80 LPH	68.52±2.02	22.70±1.16	51.96±1.62	21.55±0.93
P = 30 psi, **Q** = 120 LPH	119.64±2.84	30.45±1.32	66.84±1.73	23.53±0.88
P = 10 psi, **Q** = 120 LPH	51.84±2.06	16.98±1.06	44.52±1.74	21.69±0.97
P = 20 psi, **Q** = 120 LPH	103.68±2.46	26.14±0.98	57.84±1.89	17.95±0.63
P = 10 psi, **Q** = 40 LPH	33.24±1.19	13.92±0.66	44.52±1.88	14.58±0.59

[a] *Standard error*

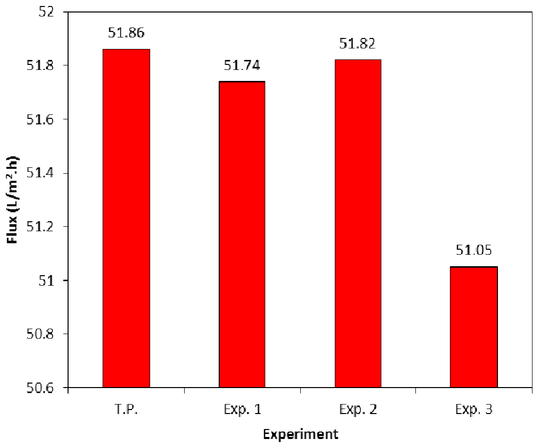

Fig. 6. Experimental results for validation of Taguchi prediction model in Marzban et al. work [75].

3.2. Ultrafiltration experiments

Tsun and co-workers [77] used the micellar-enhanced UF process to recover *thuringiensin* from the Bt fermentation broth. *Thuringiensin* is a biopesticide secreted from Bt. The chemical structure of *thuringiensin* has been measured, revealing a nucleotide analogue with a molecular weight of 701 daltons. It is a thermostable toxin known as β-exotoxin. In this work (see Ref. [77]), by using spiral-wound UF membranes (1000 and 10000 MWCO) with low-pressure and adding a surfactant, i.e. cetylpyridinium chloride (CPC) to the fermentation broth (with 4% optimal concentration), 99.3% *thuringiensin* recovery was achieved. Figure 8 shows the effect of CPC concentration on the *thuringiensin* rejection. As could be observed, with the use of the 10,000 MWCO membrane, by increasing the CPC concentration up to 3%, a sharp increase is found in *thuringiensin* rejection. In the case of 1000 MWCO, the initial rejection rate was more than 70%. With an increase in the CPC concentration up to 4%, an increase in rejection rate was observed; however, a further increase in CPC concentration had a negligible effect [77].

In another work, Tzeng et al. [78] carried out further studies on the micellar-enhanced UF process for recovery of *thuringiensin* from the supernatant of Bt fermentation broth. The authors investigated the effects of various variables, e.g. ionic strength, membrane pore size, pH, CPC concentration and temperature of micelle formation. Figure 9 shows the general concept of this work. In this work (see Ref. [78]), UF membranes with a molecular weight cut of (MWCO) 30000, 10000 and 3000, and made of cellulose acetate (CA) were used for the experiments. The effective area of all membranes was 28.7 cm^2. The pressure drop across the membrane was maintained at 207 kPa during each run. In this work, in order to study the effect of ionic strength, the supernatant samples were added with NaCl with different concentrations (i.e. 0, 1, 2, 3, 4, 5 and 6% w/v). The samples were then mixed with CPC (3% w/v). The authors used NaOH and HCl to adjust the mixture pH at 6.8, 7, 7.5, 8, 8.5 and 9. To improve the performance of micellar-enhanced UF, it is necessary to increase the amount of the *thuringiensin* to bind with micelle aggregates by introducing additional interactions. In this work, various concentrations of CPC were used. Results indicated that the *thuringiensin* recovery decreased by adding NaCl, due to neutralization of the micelle charge [78].

Among these factors, the membrane pore size and CPC concentration were the two most important factors. The authors concluded that the addition of CPC in the UF process resulted in a significant improvement in Bt recovery. The recovery increased with the initial CPC concentration in the retentate, even up to the saturation limit. On the other hand, adding inert salt, i.e. NaCl, could remarkably decrease the Bt/CPC recovery for higher ionic strength. The change in the feed stream pH was found to have no effect on the recovery efficiency [78].

Table 7
The roughness parameters and their expressions based on AFM analysis for CA and PVDF membranes with 0.22 µm pore size, in Marzban et al. work [76].

Roughness parameter	Expression	CA	PVDF
Average roughness (R_a)	$R_a = \frac{1}{n}\sum_{i=1}^{n} Z_i$	98 nm	61.6 nm
Root-mean-square roughness (R_q)	$R_q = \sqrt{\frac{1}{n}\sum_{i=1}^{n} Z_i^2}$	128 nm	77.3 nm
Peak-to-valley height (R_z)	$R_z = Z_{\max} - Z_{\min}$	771 nm	426 nm
Z_i	The height at point i		
N	Number of points in the image		
Z_{max} and Z_{min}	The highest and the lowest Z values		

Adjalle et al. [45] studied the recovery of active components (e.g. crystal proteins, spores and other factors of virulence) of Bt based biopesticides from the centrifuged supernatant, by the UF process using various membranes of 100 kDa, 30 kDa, 10 kDa and 5 kDa. The centrifuged fermented broths comprised starch industry wastewater, non-hydrolyzed and hydrolyzed wastewater sludge and semi-synthetic soya medium as control. Results indicated that the UF membrane of 5 kDa gave the highest recovery of the active components and increased the entomotoxicity in the retentates by 7.9%, 10.5%, 9.0%, 5.7%, for semi-synthetic soya medium, starch industry wastewater, non-hydrolyzed and hydrolyzed wastewater sludge, respectively. However, based on the experimental results (see Ref. [45]) it could be concluded that the retention of suspended solids on the membrane (measured via mass balance) varied with the type of fermented broths and was very high for hydrolyzed sludge (soya-15%; starch industry wastewater-12%; non-hydrolyzed sludge-7% and hydrolyzed sludge-68%). This reflected the deposit on the membrane or in other words, the membrane surface fouling. In the given context, scale-up of the UF process will give better efficacy for non-hydrolyzed sludge and starch industry wastewater in comparison to the soya and hydrolyzed sludge medium. The authors concluded that the optimum flux to achieve max total spore count in the retentate was found to be 900 $L.m^{-2}.h^{-2}$. Furthermore, there was a loss of suspended solids via deposition on the membrane surface, and the highest loss was found for the thermal alkaline hydrolyzed sludge, i.e. about 68% [45].

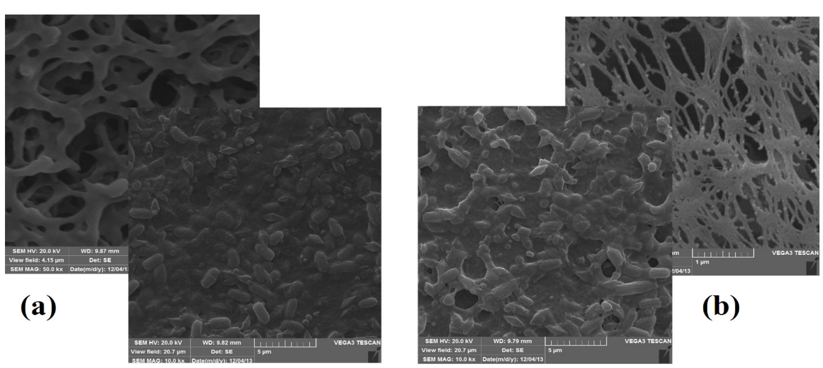

Fig. 7. SEM images of fresh and worked MF membranes, (a) CA membrane and (b) PVDF membrane both with 0.22 µm pore size, in Marzban et al. work [76].

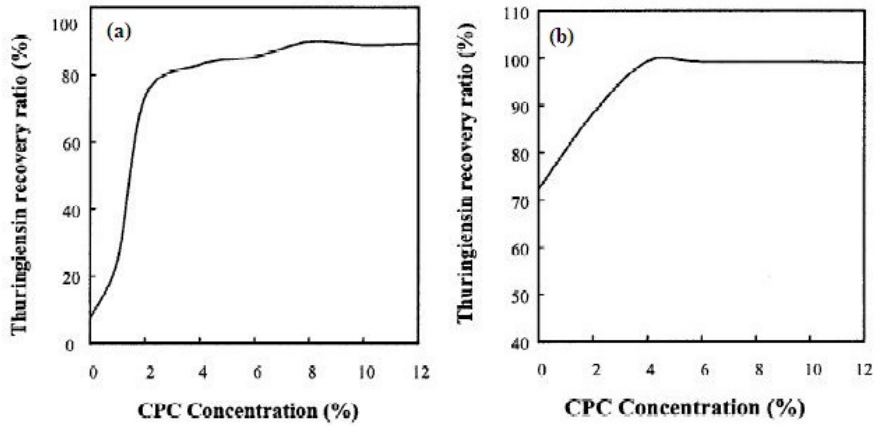

Fig. 8. Effect of CPC concentration on the thuringiensin recovery ratio after broth was filtrated with 10000 MWCO (a) and 1000 MWCO (b) membranes, in Tsun et al. work [77].

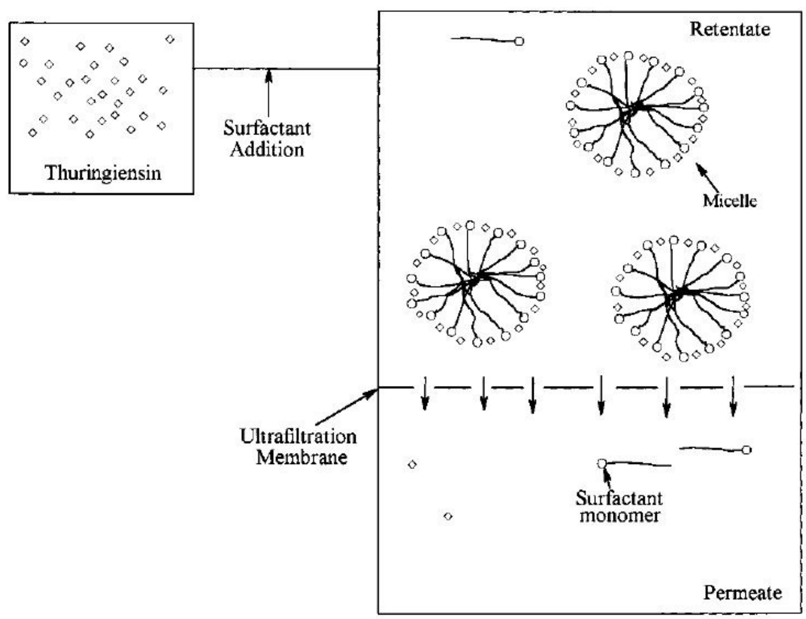

Fig. 9. A general scheme of the micellar-enhanced UF process for recovery of *thuringiensin* from the supernatant of Bt fermentation broth in [78].

Table 8
Costs analysis of three different purification method for Bt downstream processing in [44].

Method	Equipment	Costs in US$	Advantages/disadvantages
Precipitation	Not needed	Only HCl is needed	Handling of corrosive chemical
			Heavy wastage
			Toxicity and biomass yield is very less
Centrifugation	Continuous centrifuge	40000-46000	Moderate wastage
			Initial investment is high
UF filtration	UF membrane and set-up	10000-12000	No wastage
			Toxicity and biomass yield is high
			Initial investments are less than centrifugation

In a comparative study, Prabakaran and Hoti [44] investigated three different downstream processing methods, i.e. UF separation, continuous centrifugation and acid precipitation, and compared their efficiency and costs. The results of this study showed that the UF process, among others, yielded the max amount of biomass, i.e. ~53 g/L, and the max number of spores, i.e. 2.30×10^{18} CFU/mL. This was followed by continuous centrifugation and acid precipitation. Table 8 presents the cost analysis of three different downstream processing methods investigated in this work. It is to be noted that all prices are related to the date before 2008 [44].

As could be observed (see Table 8), the initial investment of the acid-precipitation is very low, but poor biomass yield and handling of corrosive chemicals are major drawbacks. Regarding the continuous centrifugation technique, the initial investment is too high and the operating and instrumentation costs are also very expensive. However, the initial investment is three to four times cheaper than that of centrifugation. On the other hand, the ease of operating conditions as well as flexibility of scale-up are other benefits of UF based downstream processing of Bt which were discussed in this work [44].

Adjalle et al. [79] investigated the scale-up of the UF process when it was used for downstream processing of Bt fermentation broth based on two wastewater samples, i.e. starch industry wastewater and hydrolyzed sludge, as well as the fermentation medium. This work reported results on the recovery of entomotoxicity components from Bt fermented broth. The study demonstrated that under optimum operating pressures of 90 and 110 kPa, the permeate flux of 550 L/m^2.h and 720 L/m^2.h were obtained for starch industry wastewater and hydrolyzed sludge, respectively. The authors reported the loss in biological activity in terms of viable spores and soluble proteins. This item was found higher for the hydrolyzed sludge due to higher viscosity and lower particle size. Moreover, in the context of scale-up, dynamic resistance can serve as a key parameter which was reported to be higher for hydrolyzed sludge when compared to starch industry wastewater. The authors concluded that the reduction in the permeate was faster for starch industry wastewater in the first 15 min [79].

4. Conclusion remarks and future perspective

Bt based biopesticidal will find wider applications in the near future by adopting simple harvesting methods and robust and economical choices of specific membranes for different downstream processing. In general, there are two types of membranes which can be used for Bt recovery from fermentation broth, i.e. polymeric membranes and ceramic membranes. The surveyed works in this study all used polymeric membranes; however, the applications of ceramic membranes have not yet been addressed. Hence, the application of ceramic membranes, which have more chemical-physical features and more reusability for spore-crystal recovery from Bt fermentation broth, particularly in large-scales, shall be investigated as future perspectives.

Various operating factors, namely feed pressure, pH, temperature, circulation velocity and membrane type influence the membrane-based recovery of Bt fermentation broth. Recently, Bt downstream processing trends have progressed; however, further studies should be carried out to enhance the penetration of Bt biopesticides into the global pesticide market. This will also greatly affect and develop the final Bt formulation, and consequently expand the repertoire of commercial Bt product types.

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