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SILKWORMS AS SUSTAINABLE PROTEIN BIOREACTORS

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ABSTRACT
Silkworms are gaining traction as cost-effective and scalable eukaryotic hosts for recombinant protein production. Their silk glands and cocoon matrices provide naturally high protein-synthesis capacity and straightforward downstream harvesting, positioning them as viable alternatives to bacterial, yeast, insect-cell, and mammalian expression systems. Recent advances in gene editing, transgenic silkworm platforms, and baculovirus-based infection strategies have enabled production of enzymes, therapeutic proteins, vaccine antigens, and engineered biomaterials with enhanced yield, folding, and functionality. At the same time, the integration of silkworm biomanufacturing with sustainable rearing approaches such as botanical pesticide formulations in insect-factory contexts offers a lower-impact production model aligned with modern green-bio manufacturing trends. This review synthesises silkworm biology and expression system design, surveys key applications and achievements, and analyses strengths, limitations and competitive placement of the system. We conclude with future-oriented perspectives on genome and synthetic biology integration, downstream process standardisation, and regulatory evolution necessary for silkworm platforms to become mainstream bioreactors.

KEYWORDS: Silkworm, Enzymes, Bio-reactor, Recombinant proteins

INTRODUCTION
Silkworms are drawing increasing attention as economical and flexible eukaryotic expression platforms for recombinant protein production. Their silk glands and cocoon matrix offer natural compartments with high protein-synthesis capacity and facile downstream harvesting routes, making them attractive alternatives to conventional expression hosts such as bacteria, yeast, insect cell lines, or mammalian cultures[1,2]. Compared with prokaryotic systems, silkworm hosts provide improved folding and eukaryotic post-translational modifications. Compared with mammalian cell culture, they require much less capital and lower running cost, and they avoid some of the biosafety and cold-chain constraints associated with large-scale mammalian fermenters[2,3].
Interest in silkworm-based biomanufacturing has grown alongside broader demands for sustainable and low-impact production systems. The life-cycle efficiency of insect rearing, coupled with potential integration of upstream (rearing) and downstream (protein recovery from silk or gland material) operations, can reduce energy and infrastructure burdens relative to industrial bioreactors[1,2]. At the same time, technical advances including improved transgenic expression methods, gene-editing tools, and scaffold/fusion strategies have increased yields and functional fidelity of complex proteins produced in silkworm systems[3,4]. These advances position silkworm-based approaches as feasible options for producing a range of bioactive proteins, from enzymes and biomaterials to vaccine antigens and therapeutic candidates[1,2].
For sericultural and insect-rearing systems, integrating biomanufacturing with sustainable pest-management practices is essential. Botanical biopesticides and green formulations, such as essential-oil based products, nanoemulsions, and other plant-derived preparations are increasingly studied as eco-friendly means to manage pest pressures while reducing synthetic pesticide residues that could compromise protein product purity or worker safety[5,6]. Reviews of biopesticides emphasize both the promise of plant-derived actives and the formulation challenges needed to obtain stability, controlled delivery, and consistent efficacy in field and rearing environments[6,7]. Thus, exploring the intersection between silkworm bioreactors and botanical, green formulation

strategies is timely. This review focuses on that intersection, summarizing silkworm expression platforms and recent technical innovations, examining relevant botanical pesticide and green-formulation approaches that could support sustainable rearing, and discussing the practical and regulatory considerations for integrating these elements into scalable, low-impact recombinant protein production workflows.

Silkworm Biology and Its Potential as a Bioreactor

Silkworms possess several biological and practical features that make them attractive eukaryotic hosts for recombinant protein production. Recent high-quality genome assemblies and pan-genome resources have clarified the genetic architecture that underlies silk synthesis and other metabolic processes, providing a foundation for targeted genetic manipulation and strain improvement. These genomic resources reveal large, well-annotated repertoires of genes expressed in silk-producing tissues, and they enable discovery of regulatory elements and allelic variation useful for optimizing expression of heterologous proteins[8,9].

The silk gland itself is a naturally specialized protein-production organ with extremely high synthetic capacity and an intrinsic secretory pathway. Single-cell and transcriptomic studies have mapped the cellular composition and cell-type specific expression programs of the silk gland, demonstrating that particular gland cell populations are dedicated to massive synthesis, folding, and secretion of structural proteins[10]. Molecular and developmental studies further show that endoreplication and cell-cycle regulators expand biosynthetic capacity in gland cells, linking developmental control to large-scale protein output. These organ-level specializations make silk glands ideal biological "factories" for expressing both native and exogenous proteins, because they combine high translational throughput with native secretory and post-translational processing pathways[10,11].

Modern gene-editing and transgenic approaches have made it possible to harness the silk gland (and other silkworm tissues) as expression sites for diverse recombinant products. Precise genome assemblies and editing tools, including homologous-recombination mediated strategies and CRISPR/TALEN methods now permit insertion of expression cassettes into endogenous loci or fusion to silk protein carriers, yielding high yields and simplified downstream recovery from cocoon matrices or haemolymph[4,12]. In parallel, baculovirus-based expression systems adapted to whole silkworm larvae or pupae remain an efficient route for rapid, high-yield production of secreted and complex proteins, and have been used successfully for vaccine antigens, antibodies, and virus-like particles[13,14].

From an applied perspective, silkworm rearing is scalable and relatively low-cost when compared with large mammalian bioreactors. The coupling of simple husbandry, short generation times, and high per-animal protein output supports modular production models in which rearing and initial recovery (e.g., gland excision or cocoon harvest) integrate with downstream purification pipelines[13,15]. Moreover, advances in strain genomics and husbandry inform selective breeding or genome-assisted selection strategies to maximize yield, product quality, and robustness under controlled rearing conditions[8,9]. Taken together, these biological, genetic, and operational attributes make silkworms compelling candidates as sustainable, versatile bioreactors for a broad range of recombinant proteins.

Recombinant Protein Expression Systems in Silkworms

Two principal platforms have been developed to exploit silkworms for heterologous protein production: baculovirus-mediated expression using insect-specific baculovirus vectors, commonly referred to as BEVS when used in insect cells and adapted for whole-animal silkworm expression, and stable transgenic expression in silkworm tissues, especially the silk glands. Both approaches have complementary strengths, BEVS delivers rapid, high-level transient expression suitable for screening and short-term production runs, while transgenic strategies enable stable, heritable expression and continuous harvesting from silk or gland material[13,16].

Baculovirus-mediated expression exploits recombinant baculoviruses engineered to carry target genes under strong viral promoters and to infect silkworm larvae or pupae. Adaptations of BEVS for whole-organism silkworm production have achieved high yields of secreted and membrane proteins, virus-like particles, and vaccine antigens, with efficient folding and biological activity in many cases[13,17]. Recent technical improvements include optimized bacmid editing to remove nonessential viral genes, insertion of stabilizing elements, and refined timing and routes of infection to maximize expression while minimizing host morbidity. These advances have increased per-animal yields and simplified downstream recovery from haemolymph or cocoon matrices[12,13].

Transgenic silkworm approaches insert expression cassettes into the host genome to drive tissue-specific and often constitutive production of recombinant proteins. A widely used tactic is fusion of heterologous proteins to endogenous silk protein carriers, which concentrates the recombinant target into silk fibers or gland secretions and facilitates simple mechanical recovery from cocoons[4,14]. Stable genomic insertion has been improved through targeted editing tools. TALEN-mediated homologous recombination and other precise genome-editing strategies have enhanced insertion efficiency and expression stability, resulting in higher, heritable yields and predictable expression patterns[4,12].

Yield, folding, and post-translational modification (PTM) of complex proteins are active areas of innovation. To improve folding and solubility, researchers have implemented fusion tags, chaperone co-expression, and ER-retention signals to harness the endogenous secretory pathway of silk gland cells[13,17]. For PTMs, especially N-glycosylation that is critical for many therapeutics, insect systems historically present simpler glycan structures

than mammalian hosts; recent glycoengineering strategies in insect expression platforms, such as heterologous expression of mammalian glycosyltransferases, inducible glycogene expression, and host-cell pathway remodeling have narrowed this gap and produced more "mammalian-like" glycosylation on recombinant products[16,18]. These glycoengineering advances, while more mature in cultured insect cells, are being ported to whole-organism silkworm workflows through baculovirus vector design and host modification, improving the functional fidelity of complex glycoproteins produced in silkworm systems[16,18].

Finally, vector and host engineering have together enabled increased production robustness and product quality. Improvements in bacmid editing and vector design permit modular swapping of promoters, signal peptides, and stabilizing domains to tune expression kinetics and secretory efficiency[12,16]. Concurrently, advances in genomic resources and gene-editing tools for silkworms permit strain development focused on yield, reduced proteolysis, and enhanced tolerance to infection or harvest procedures[4,17]. Collectively, these gains are moving silkworm expression platforms from demonstration studies toward scalable, quality-driven production options for vaccines, enzymes, and other recombinant biologics.

Applications and Achievements in Recombinant Protein Production

Silkworm-based expression platforms have produced a wide range of recombinant proteins with clear real-world relevance.

Enzymes

Silkworm expression systems have been used to produce active enzymes for biochemical and industrial use. Enzymes produced via baculovirus–silkworm or transgenic approaches often show correct folding and activity, and their recovery from haemolymph or cocoon material simplifies purification for secreted enzymes[17,19]. Work on expression and biochemical characterization of enzymes such as endo-β-N-acetylglucosaminidase and other hydrolases demonstrates that the system can yield enzymatically active proteins at useful scales, making silkworms an at tractive host for specialty enzyme production[20].

Pharmaceuticals (therapeutic proteins and antibodies)

Silkworms have produced functional therapeutic proteins and monoclonal antibodies using both stable transgenic expression and baculovirus-mediated methods. Early and mid-decade demonstrations include expression of monoclonal antibodies with retained antigen binding and effector properties from transgenic cocoons[21]. More recent genome-engineering and expression-optimization work has improved yields and quality control, moving these products closer to therapeutic-grade material and supporting the notion that silkworms can serve as low-cost production platforms for certain biologics[4,17].

Vaccines and vaccine antigens

Vaccine antigen production is one of the most compelling real-world successes for silkworm systems. Baculovirus-silkworm workflows have been used to produce virus-like particles and viral surface proteins for immunization studies and subunit vaccine development. Notably, SARS-CoV-2 spike protein and receptor binding domain constructs were efficiently expressed in silkworm larvae/pupae with demonstrated antigenicity and utility in serological assays and immunization experiments[14,22,23]. The system has also been applied to norovirus and other viral antigens, highlighting its flexibility for rapid vaccine antigen production during outbreaks.

Industrial and biomaterial proteins (silk modification and spider silk)

Silkworms are uniquely suited to produce engineered silk and silk-fusion products at scale. Transgenic strategies that incorporate heterologous proteins or spider-silk motifs into silk fibers enable production of high-value biomaterials with tailored mechanical and functional properties[24,25]. These engineered silks serve industrial and biomedical ends from high-strength fibers to scaffolds for tissue engineering and the native silk-production organ simplifies downstream processing compared with other host systems. Collectively, these examples illustrate the versatility of silkworm-based systems in producing recombinant proteins with industrial, pharmaceutical, and biomedical significance. A concise summary of representative proteins expressed using silkworm platforms is presented in Table 1.

Table 1: Major recombinant proteins produced using silkworm-based systems

Product type	Protein	Expression method	Outcome	Reference
Enzyme	Endo-β-N-acetylglucosaminidase H	Baculovirus–silkworm	Active enzyme recovered from haemolymph	Mitsudome et al., 2014
Therapeutic protein	Anti-CD20 monoclonal antibody	Transgenic silk gland	Functional antibody with correct binding	Tada et al., 2015
Vaccine antigen	SARS-CoV-2 spike/RBD	Baculovirus–silkworm	Correct folding, immunogenic, used in vaccine trials	Fujita et al., 2020; Masuda et al., 2022

Biomaterial	Drosophila Dumpy fusion silk	Transgenic cocoon silk	High mechanical strength fibers	Dai et al., 2024
Spider silk protein	Spider dragline motifs	Transgenic silkworm silk	High-strength silk fibers	Ramezaniaghdam et al., 2022

Advantages, Limitations, and Comparative Perspectives

Silkworm expression systems combine biologically relevant eukaryotic machinery with practical, low-infrastructure scalability, creating a niche between simple microbial hosts and capital-intensive mammalian cell culture. Biologically, silkworms provide authentic secretory pathways and many eukaryotic post-translational processes, while the silk gland is a naturally high-capacity protein synthesis organ that concentrates products into secreted matrices amenable to mechanical recovery[13,17]. Genomic resources and improved gene-editing methods enable targeted host modifications and reliable insertion of expression cassettes, which together increase the predictability and potential yield of heterologous proteins[4,8]. These attributes make silkworms particularly suitable for secreted enzymes, biomaterials, and certain vaccine antigens where eukaryotic folding and secretion are important but full mammalian PTM fidelity is not strictly required.

Economically and operationally, silkworms can lower capital and running costs relative to mammalian bioreactors. Rearing infrastructure is simple and modular, scaling is achieved by adding animals rather than enlarging fermenters, and when products are concentrated in cocoons or gland tissue, initial downstream steps (harvest, mechanical separation) can be simpler than cell-culture harvests. This cost and infrastructure profile can favor regional or decentralized production models and small-to-medium production volumes where the economics of mammalian culture are unfavorable[13,26].

Nevertheless, silkworm systems present technical challenges that affect product quality and regulatory acceptance. Purification from complex insect tissues or cocoon matrices can co-extract host proteases, pigments, and other contaminants, necessitating robust multi-step purification to reach pharmaceutical grade[13,17]. In addition, native insect N-glycosylation tends toward simpler paucimannose structures and lacks many mammalians terminal modifications; this can alter protein activity, half-life, or immunogenicity for some therapeutics[18,27]. While glycoengineering strategies (CRISPR editing of glycosylation enzymes, heterologous expression of mammalian glycosyltransferases, inducible glycogene systems) have advanced substantially in insect cell cultures and model insect lines, porting these reliably to whole-animal silkworm workflows remains an ongoing area of development[16,18,27].

Regulatory and biosafety considerations are another major axis. Whole-animal production using baculovirus vectors or transgenic animals raises issues of process standardization, potential residual viral particles or host nucleic acids, cohort variability, and the need for validated containment, inactivation, and impurity-clearance steps that meet regulatory expectations for biologics. Demonstrating consistent batch-to-batch quality and implementing GMP-level controls for rearing, feed, and pathogen monitoring will be essential for therapeutic applications; these are areas where mammalian cell systems currently have an established regulatory pathway and precedent[17,28].

When compared with other major expression platforms, silkworms have clear trade-offs. Bacterial hosts (e.g., E. coli) are fastest and cheapest for simple, non-glycosylated proteins but fail for complex folding and eukaryotic PTMs[29,30]. Mammalian cell systems (e.g., CHO) provide human-like glycosylation and are the regulatory standard for most therapeutics, yet they demand expensive infrastructure and operating costs[28,29]. Insect cell/Baculovirus Expression Vector Systems (BEVS) in suspension bridge the two: they offer eukaryotic folding with moderate PTM capability and are amenable to glycoengineering in well-controlled bioreactors[16,27]. Silkworm whole-organism systems share many advantages of insect BEVS (eukaryotic processing, lower cost) and add the silk gland's natural concentration and low-tech scalability, but they currently lag behind in process standardization, regulatory precedent, and some glycoengineering maturity. The optimal platform therefore depends on product requirements. For fully humanized glycoforms and highest regulatory confidence, mammalian systems remain preferred. For rapid, cost-sensitive production of antigens, enzymes, or silk-fused biomaterials, silkworms are an attractive alternative[16,27,30].

Silkworm-based systems thus represent a balanced expression platform combining eukaryotic processing fidelity with cost-effective, scalable production. Although challenges remain, particularly in purification, native glycosylation profiles, and regulatory standardization, ongoing advances in host engineering, vector optimization, and process control continue to narrow these gaps. Selecting an appropriate production host should consider the protein's structural requirements, scale of production, and intended regulatory pathway to leverage the distinct advantages of the silkworm system[13,16]. A comparative summary of key attributes across major recombinant protein expression systems, including silkworm-based platforms, is presented in Table 2.

Table 2: Comparative overview of recombinant protein expression systems

Expression system	Host type	Typical yield	PTM capability	Production cost	Scalability	Major limitations

E. coli	Prokaryote	Very high	None	Very low	High	Misfolding, inclusion bodies, lacks PTMs
Yeast	Eukaryote	High	Basic glycosylation	Low	High	Hypermannosylation, limited folding fidelity
Insect-cell BEVS	Eukaryote	Moderate	Moderate (insect-type glycans)	Medium	Moderate	Complex setup, needs bioreactors
Mammalian cells (CHO/HEK)	Eukaryote	Moderate-high	Full mammalian PTMs	Very high	Limited	High cost, long culture time
Silkworm larvae/pupae	Whole insect	High	Eukaryotic (partial mammalian-like)	Low	Easy to scale	Glycan differences, regulatory hurdles

Future perspectives

To unlock the full potential of silkworms as scalable, sustainable bioreactors, efforts should prioritize (1) precision host engineering to tailor biosynthetic capacity and reduce proteolytic or contaminant burdens; (2) transfer and adaptation of glycoengineering and secretory-pathway modifications from insect-cell platforms into whole-animal workflows to produce therapeutically relevant glycoforms; (3) expanded synthetic-biology toolkits (including genetic-code expansion and RNA-editing) to permit site-specific modifications and conditional control of expression; and (4) development of standardized, GMP-compatible rearing, harvest, and impurity-clearance pipelines to meet regulatory expectations for biologics. Precision insertion and locus control (e.g., TALEN/HR strategies) and single-cell insights into gland cell types enable rational targeting of high-yield expression sites and reduction of off-target host effects[4,10]. Glycoengineering and vector/host optimization advances demonstrated in BEVS and insect-cell systems provide a clear roadmap for improving PTM fidelity in whole-animal production[16,27]. New tools from synthetic biology such as genetic code expansion to introduce non-canonical amino acids and CRISPR-based RNA editors will broaden functionalization options for novel biomaterials and therapeutic constructs expressed in silkworms[31,33]. Finally, integrating these molecular advances with validated downstream purification workflows and robust process-control (feed, pathogen monitoring, inactivation of vector residuals) is essential to translate laboratory proofs-of-concept into regulated, industrial-scale production[3,13].

CONCLUSION

Silkworm-based expression platforms occupy a strategic niche in recombinant protein production, blending the fidelity of eukaryotic expression with the scalability and lower infrastructure demands of insect-based systems. Their unique biology such as the large protein-synthetic capacity of silk glands, ease of rearing, and ready integration into harvesting workflows makes them well suited for enzymes, vaccine antigens, biomaterials and other proteins where complex post-translational modification, moderate scale, and cost-sensitivity intersect. However, realising this potential at industrial scale requires concerted progress in several domains: streamlining purification from complex insect tissues, bridging the glycosylation and PTM gap between insect hosts and mammalian systems, and navigating regulatory pathways for whole-organism bioreactors. With continuing innovations in host engineering, vector design and process control, silkworms can evolve from demonstration systems into full-fledged biomanufacturing platforms enabling lower-cost, distributed production of next-generation biologics and functional proteins.

Conflict of interest

The authors declare no conflict of interest.

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Authors' contributions:

The work was carried out in contribution among all authors. Author PP designed the study and wrote the first draft of manuscript. Author PP wrote the protocol and perform the statistical analysis. Author AK, TA, MM, KA, PG, RS, JS and PN managed work and performed the corrections in the manuscript.

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