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Abstract:	Despite the advantages of plant-based transient expression systems relative to microbial or mammalian cell systems, the commercial production of recombinant proteins using plants has not yet been achieved to any significant extent. One of the challenges has been the lack of published data on the costs of manufacture for products other than biopharmaceuticals. In this study, we report on the techno-economic analysis of the production of a standard commercial enzyme, namely horseradish peroxidase (HRP), using a transient expression system in Nicotiana benthamiana. Based on the proven plant yield of 240 mg HRP/kg biomass, a biomass productivity of 15 kg biomass/m2/year and a process yield of 54% (mg HRP product/mg HRP in biomass), it is apparent that HRP can be manufactured economically via transient expression in plants in a large scale facility (> 5 kg HRP/year). At this level, the process is competitive vs. the existing technology (extraction of the enzyme from horseradish) and the product is of comparable or improved activity, containing only the preferred isoenzyme C. Production scale, protein yield and biomass productivity are found to be the most important determinants of overall viability.

Introduction

Recombinant protein expression in plants

The use of plants for the production of high-value recombinant proteins has been explored for over 20 years. Numerous strategies have been followed, broadly categorised into stable and transient expression (19), the advantages and disadvantages of which have been reviewed recently (24).

Briefly, transgenic expression entails stable transformation of the nuclear genome with the gene of interest and a promoter; the gene is then expressed constitutively, or after induction using a specific agent. Genes may be expressed throughout a plant, or limited to specific tissues by choice of a specific promoter. A special case of transgenesis is in transplastomic plants or cells, where only the chloroplasts have been transformed. As long as expression is stable and at a reasonable level, transgenesis is the cheapest means of producing recombinant proteins in plants – with the only real drawbacks being the many months required to transform, regenerate and bulk up transgenic plants, and possibly low yields for certain proteins..

Transient expression involves the somatic introduction of foreign genes into plants or cell cultures by means of some kind of vector. This is preferentially via recombinant *Agrobacterium tumefaciens*: a bacterial culture can be co-cultivated with cultured cells, or injected directly into the abaxial air spaces of whole plant leaves (very labour intensive and impractical for large scale production), or introduced by submerging trays of plants in the inoculum solution, extracting the abaxial air under vacuum, and then replacing this air with the *Agrobacterium* suspension. In all these cases, transfer of T-DNA from *Agrobacterium* into plant cells results mainly in episomal rather than

integrated DNA, from which expression can occur in all of the cells so transfected. This process has been optimised for large scale expression by several companies such as Kentucky BioProcessing and Medicago Inc.

Plant virus-derived vectors may be useful in increasing yields and these vectors may be used alone. However, they are often limited in terms of stability and ability to spread, and combining virus-derived DNA and cDNA vectors with agroinfiltration is currently the state of the art for high-level expression (12).

Transient expression has the advantage of significantly faster protein production timelines and improved protein accumulation (5). Although initially hindered by the lack of a scalable gene-delivery technology, vacuum infiltration has overcome this challenge, and transient expression is now widely used as robust and efficient system for the transient expression of high-value recombinant proteins in plants (24).

The technology is difficult to develop but relatively simple to apply once it has been optimised for protein expression (9, 16) and yields are often superior to those from transgenic plants (28). The main factor in the development is to identify the most suitable vector system that will induce high levels of high purity protein production, whilst maintaining plant viability over a period of between 3 to 10 days, which is the typical incubation time for transient expression. The actual incubation time depends on the vector system employed and the heterologous protein being expressed, and needs to be optimised experimentally for each system (22, 21).

Despite a number of advantages of plant expression systems relative to microbial or mammalian cell culture, including the lack of human or animal pathogens, low upstream costs and high scalability (20, 25), the commercialisation of plant-made biopharmaceuticals and other proteins has lagged the alternative systems (27). However there have been several recent breakthroughs, with the first plant-based human therapeutic (taliglucerase alfa for Gaucher disease; Protalix, Israel) being approved in 2012 by the Federal Drug Administration (FDA) (10), and InVitria's recombinant human lactoferrin being marketed commercially as a laboratory reagent (15). There is now renewed optimism that plants can indeed be used as an alternative to microbial fermentation and other protein-production systems – and for broad-based commercial products, rather than only for very high-value vaccines and therapeutics.

A key consideration, which remains to be finally validated, is the economic viability of plant-based protein production. Despite repeated claims in the literature about the cost benefits of plants as hosts for the manufacture of commercial proteins (8, 26), there are few published studies covering the techno-economics of such systems (3, 4). Buyel and Fischer (4) report on a cost model to identify suitable optimisation strategies for the production of recombinant protein *via* transient expression in *N. benthamiana*. Broz *et al.* (3) describe InVitria's programme to commercialise human lactoferrin production in rice; expression levels of 6 g protein per kg seed were obtained and a target cost-of-goods-sold of $3.75 per gram lactoferrin was "comfortably achieved".

A recent unpublished feasibility study undertaken by Hendricks and Thiel (13) noted that plant-based protein production has the potential to become a commercially attractive and universally accepted approach for vaccine and therapeutic manufacturing. However,

they also stated that the "key economic driver is the return on R&D, primarily as a result of the substantial investment required in clinical development" and that the "overwhelming driver to significantly reduce production costs lies in the economy of scale". The report concluded that the establishment of a biopharming platform will require public sector subsidisation of R&D costs and the selection of a high-volume product with a "well-crafted market entry strategy".

These conclusions are clearly relevant specifically to human therapeutics; the study did not assess the production of commercial enzymes or animal therapeutics, for which the issue of clinical development is either not applicable or less costly, and as a consequence the economic viability of plant-based protein production in these two significant market areas remains an open question.

Accordingly, in this study, we examined the techno-economics of the commercial production of an important and well-known commercial reagent protein, namely horseradish peroxidase (HRP), based on a study of agroinfiltration and transient expression of the recombinant enzyme in *Nicotiana benthamiana* plants (14).

Overview of Horseradish Peroxidase

Horseradish is a perennial rootcrop of the *Cruciferae* family. It is one of the oldest known condiments, valued for its extremely pungent, fleshy roots (18). The bulk of the horseradish is processed into a paste and used as a food additive; its pungency is due to the hydrolysis of sinigrin, which is present in the fresh radish root, by the enzyme myrosinase into allylisothiocyanate, butylthiocyanate, glucose, and sulphate.

Allylisothionate is highly volatile and easily vaporises from the mouth into the nose, where it triggers the well-known burning sensation.

Horseradish roots are rich in horseradish peroxidase (HRP), the latter being an alpha-helical glycoprotein which catalyses the oxidation of various substrates in the presence of hydrogen peroxide. There are a large number of isoenzymes, with the most prevalent being the C type (29). About 80% of the commercially available enzyme, all of which is presently obtained directly from the plant, is used in kits to test for levels of glucose and cholesterol in blood. Other applications include bio-bleaching, waste water treatment, oxidase-based immunoassays, enzyme activity assays, cytochemistry and preparation of DNA probes.

While commercial enterprises generally extract HRP directly from horseradish roots, there are a number of disadvantages to this route including the constraint that all the isoenzymes (including unwanted isomers) are co-purified; the roots are only available seasonally (requiring large on-site refrigerated storage); and modification of the enzymes is only possible by chemical means post-expression in the natural plant material. Accordingly, Huddy *et al.* (14) explored the feasibility of using agroinfiltration of *Nicotiana benthamiana* under vacuum as a means of producing a single isotype of HRP. The attempt was successful, resulting in an expression yield of HRP isotype C at high levels (240 mg per kg biomass in the unprocessed plant material).

Published information on the global market for horseradish peroxidase is inconsistent and appears to be unreliable. In 1991, the global market for HRP was estimated at 30 million kilo-units, with demand expecting to double by 2010 (18, 17). A more recent

report estimates that the total market for oxidoreductases, which includes HRP and glucose oxidase, was about $65 million in 2011 (7). The study by Barnard (2) reported that the global company BBI Solutions produces about 7 billion activity units (AUs) per annum and holds a 20% market share, making the total market about 35 billion AUs.

The product is sold as a lyophilised powder at an enzyme activity of between 50 and 330 AU/mg; the price per kilo-unit is highly variable, depending on the quality, the enzyme type and the quantity. Prices from various suppliers for both horseradish and HRP are shown in Table 1; the bulk of the enzyme is supplied at 250 AUs/mg.

Assuming an average activity of 250 AUs/mg, a global market of 35 billion AUs and an average price of $5,000/AU (or $1,250/g HRP), the total global market value and volume are estimated at $210 million and 140 kg HRP respectively. However the average price is difficult to calculate accurately since volumes for specific grades are unknown; the value of $5,000/AU or $1,250/g is obtained from the middle range of the prices for bulk HRP (orders > 1 g) as shown in Table 1. Although this could not be confirmed, it is suspected that many buyers in the market prefer a high quality product in small quantities (< 10 mg).

Existing manufacturing technology: extraction of roots

HRP is traditionally obtained via extraction from horseradish. Yields of enzyme per unit weight of radish are highly variable and depend on climatic and other factors. In South Africa much of the horseradish is grown in the Eastern Cape, the bulk biomass from this region having higher HRP content and a lower concentration of the gelatinous material that often interferes with the downstream filtration of the plant extract. The existing

Table 1. Market prices for horseradish root and horseradish peroxidase

Supplier	Product and Grade	Price Units	Activity (AU/mg)	Price According to Quantity of Order		
				1 g	1 kg	1 MT
Rizhao Huamei Foodstuffs	Horseradish root (fresh, topped)	$/kg	N/A	N/A	N/A	1.06
Xinghua Shengyuan Foods Co	Horseradish root (dry, milled)	$/kg	N/A	N/A	N/A	2.55
Various suppliers	Enzyme (crude)	$/g	150	270	2.7	N/A
	Enzyme (medium)		250-330	200 - 320	2.65	N/A
	Enzyme (high)		>330	400 – 3,750	N/A	N/A

South African HRP manufacturers - BBI Enzymes, formerly Seravac Biotech and now part of the BBI Enzymes group based in Wales, and Faizyme - purchase the bulk radish directly from farmers, the latter growing the product under contract at a fixed purchase price.

Information on the contracts, the HRP content and quality, purification efficiencies and radish volumes is not publicly available; however the following has been established from various sources:

- HRP concentration in horseradish root varies from 56 mg/kg (18) to 156 mg/kg (2) depending on the source and season; in South Africa, values of 156 mg/kg may be more typical (2)
- biomass is purchased from local farmers at $1 to $1.30 per kg, which is equivalent to about $7/g HRP and represents only a small fraction of the overall value of the product (< 1%)
- the commercial extraction and purification efficiency of pure HRP from crude biomass is about 20%, giving an overall enzyme yield of about 32 mg/kg biomass; in other words, the cost equivalent of biomass to the refiner is $36/g HRP
- the average extraction cost for HRP of activity between 250 and 300 AU/mg is between $300 and $1,500/g HRP, depending on the grade (the higher the purity, the higher the cost) and plant capacity.

For this study, it was assumed that a new technology will need to achieve a price target of no more than the average price of $1,250/g HRP at a plant capacity of about 10 kg HRP per year and an enzyme purity of at least 250 kU/g HRP. These values have been

used in the remainder of this article in order to assess the competitiveness of the transient expression technology for HRP production.

Materials and Methods

Financial models

Estimates for the cost of production for HRP have been made using two different financial models namely, a single year costing (SYC) technique, and a discounted cash flow (DCF) algorithm. The SYC approach estimates the total cost based on a fully absorbed cost of production, including total direct costs (raw materials, direct labour-production and quality control), indirect fixed costs (indirect labour, maintenance, distribution and warehousing, sales and marketing), royalty and finance costs (working capital charges and return on capital invested). Average values as used in this study for HRP are shown in Table 2, with more details provided in the Supplementary Material.

The DCF algorithm uses the same estimates for raw material cost but calculates a net present value (NPV) and internal rate of return (IRR) based on the components of the fully absorbed cost (capital, fixed and variable costs).

In both cases the raw material costs have been estimated from a mass balance for the process developed from the laboratory description, and a number of standard financial values have been assumed, some of which can vary according to the country in which the manufacturing facility is located (such as the cost of capital). In this study, values specific to South Africa have been used; further details on each item and ranges for the values have been provided in the Supplementary Material.

Table 2. Base case assumptions for financial modelling

Parameter	**Units**	**Value**
Exchange Rate	ZAR/$	10
Discount Rate	% p.a.	8
Single Year Return on Investment	% p.a.	20
Depreciation Rate	% p.a.	10
Overhead Costs (Direct Labour)	% of TCOE	50
Overhead Costs (Indirect Labour)	% of TCOE	110
Working Capital Factor	% p.a.	10
Maintenance Factor	% p.a.	5
Distribution and Warehousing Factor	% p.a.	5
Sales and Marketing Factor	% p.a.	5
Royalty	% p.a.	5

Estimation of capital cost

The capital cost is calculated from cost of the greenhouse plus the processing facility's main plant items, the latter being obtained from a process design for the facility and listed on its process flow diagrams. The capital cost is then multiplied by a series of roll-up factors to derive the total installed cost (see Table 3). This is a standard approach in the process industries and leads to a capital cost estimate with a standard deviation of ±30%, which is usually adequate for early stage projects with sparsely-defined flow sheets (1, 6).

In addition to these algorithms, the analysis also draws on the important characteristic of pharmaceutical production and the process industries; namely, that equipment prices per unit of capacity scale in proportion to size, observing the correlation of:

$$C \qquad \left(-\right)$$

where C_a is the equipment cost of production at capacity Q, and C_b and Q_b are the base costs and capacities respectively (23).

The greenhouse cost is a specific high cost item around which there is some uncertainty due to mixed opinions on the required technical specifications. A fully equipped state-of-the-art greenhouse, with total containment and/or sterilisation of all input and output process in order to prevent the possibility of leakage of genetically modified organisms to the environment, will cost up to \$5,000 per m^2 depending on the degree of biocontainment and mechanisation (13). However such a facility will be over-engineered

Table 3. Roll-up factors used for estimation of total capital cost

Item	**Cost ($)**
Greenhouse	A
Main Plant Items (Processing Plant)	MPI
Building	MPI*.33
Utilities	MPI*.26
QA Labs	MPI*.12
Consultant and Engineering Fees	MPI*.2
Contingency	MPI*.28
Total Capital Cost	A+MPI*1.19

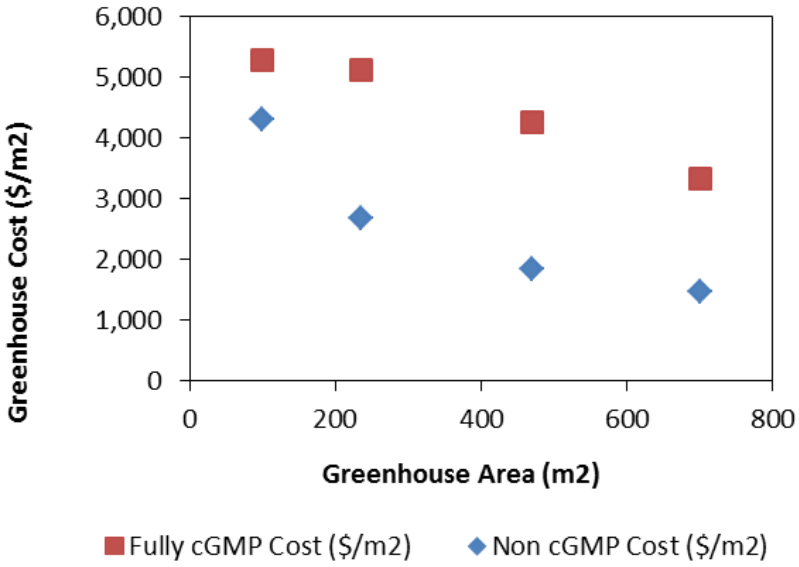
for the production of a commercial enzyme such as HRP. For this study, the following approach was adopted:

- the greenhouse was considered to be a flexible structure which can be separated into pre- and post-infiltration, and as many single production trains as necessary to accommodate one complete growth and infection batch cycle
- it was assumed that the pre-infiltration plants can be accommodated in a lower cost facility, for which the average installed cost would be \$500 per m^2, depending on the size of the greenhouse; the latter value is still inflated relative to the price of a 'high cost' greenhouse as used in commercial agriculture since it includes additional mechanisation features not normally fitted in the latter facilities (11)
- post-infiltration plants will only be grown in a more elaborate facility, whose average cost was assumed to be \$2,000 per m^2, depending on the size of the greenhouse; this value was obtained from the correlation as shown in Figure 1 (13), which depicts the costs of greenhouses as required for the production of human therapeutics (cGMP) and 'non cGMP' applications, where the latter may include enzymes and reagents requiring a less stringent degree of isolation, containment, mechanisation and waste processing.

The required area for the greenhouse was calculated using the assumptions of:

- each plant is located in a 2" (5 cm) cell, with 24 cells per tray, giving a total plant and tray footprint of 0.003 and 0.06 m^2 respectively
- the facility operates 365 days per year, and the greenhouse usable area is 70%

Figure 1. Fully cGMP and non-cGMP greenhouse costs



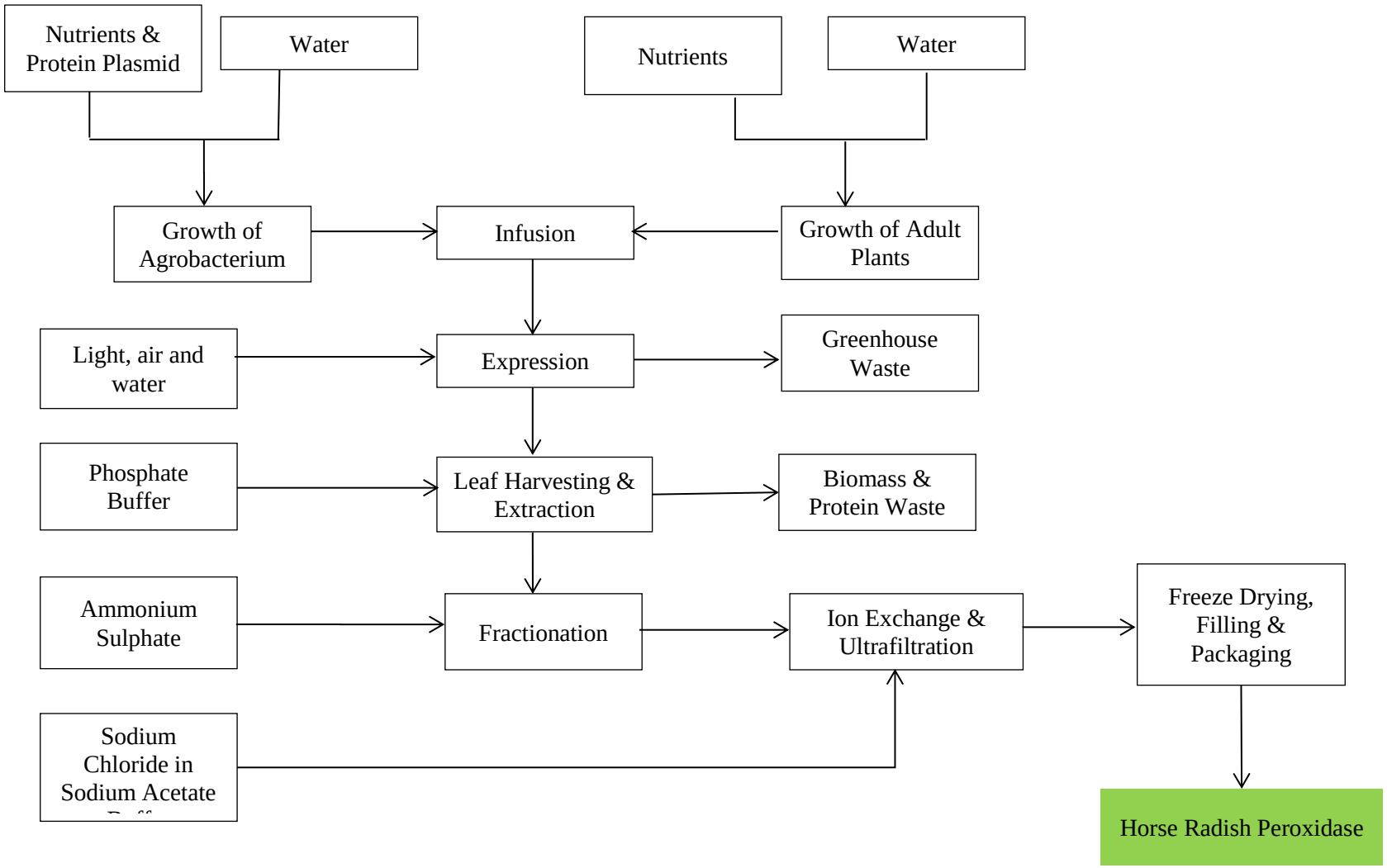
- the expression time is variable depending on the process requirements (7 to 10 days)
- for HRP, the overall growth time is a total of 45.5 days including 7 days for seedling growth, 35 days for pre-infection growth and 3.5 days turnaround (mainly in the post-infiltration greenhouse).

Sensitivity analysis

Relative sensitivity analyses are often conducted as part of techno-economic studies, but these analyses are frequently misleading as a consequence of a normalisation problem. Although the intention may be to establish the relative impact on the dependent variable (in this case the fully absorbed cost or the project NPV) of equally-likely changes in the key input variables, it is almost impossible to establish the magnitude of such changes across different variables. In other words, although the analysis is intended to identify those input variable(s) that should be targeted in order to achieve the maximum improvement in the dependent variable(s), in practice such evaluations are mostly undertaken without any consideration of the relative difficulty of achieving the proposed increase in a specific input variable. For instance, doubling the protein yield may be more difficult than a doubling of the greenhouse capacity or a 50% reduction in capital cost.

Nevertheless, in this study, the standard approach was followed. All input variables were initially screened in order to identify those variables for which a 20% change in value had a dominant impact on the fully absorbed cost. The impact of the top 5 variables were then further analysed in order to generate the sensitivity profiles as reported later in this article.

Figure 2. Block flow diagram for HRP production



Manufacturing process

HRP can be also produced using the infiltration technology as described earlier (14). The process requires the separate preparation of the *Agrobacterium* inoculum and adult *Nicotiana benthamiana* plants; the latter are infiltrated under vacuum with the inoculum and allowed to grow/express protein for a further 7 days. The HRP-enriched biomass is harvested, homogenised in ice-cold 0.1 M potassium phosphate buffer and centrifuged to remove the biomass. The crude protein extract is further fractionated with ammonium sulphate and finally purified using ion exchange chromatography before being lyophilised and packaged (see Figure 2).

Results

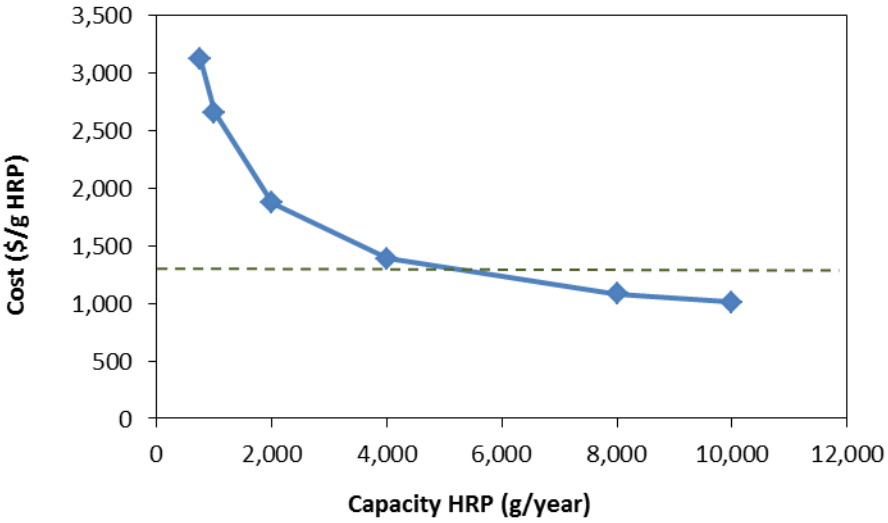
Techno-economic assessment

For the techno-economic assessment, we consider a base case scenario and two alternative arrangements. The base case is taken as a capacity of 5 kg HRP per year and the growth of adult plants from seeds, with only the incubation part of the greenhouse area being certified for GM crop production. The two alternative arrangements are buying adult plants directly at $0.82/plant (Alternative 1), and the use of a greenhouse fully certified for the whole area (growth of adult plants and incubation; Alternative 2). Table 4 shows a high level summary of the techno-economic results for the base case and the two alternative scenarios. It is noted that the DCF model is more conservative than the SYC model under the same inputs on account of the more conservative assumptions for the revenue streams (the DCF model allows a ramping of production capacity in the initial period, which reduces cash flows and hence the NPV).

Table 4. Results of techno-economic assessments for base case and alternatives

Scenario	**Units**	**Base Case**	**Alternative 1**	**Alternative 2**
Fully Absorbed Cost (SYC)	$/g HRP	1,279	2,489	1,429
NPV (DCF)	$ mill	-6.1	-36.477	-10.156
IRR (DCF)	(%)	1.7%	<0%	<0%

Figure 3. The fully absorbed cost ($/g HRP) depends on production capacity (dashed line is the HRP selling price)



It is clear from Table 4 that all assessments have resulted in a negative NPV or a rate of return which is below the cost of capital; in other words, the process is presently uneconomic for enzyme production capacities below 5 kg HRP/annum. Although the source of the adult plants (grow or buy) and the greenhouse configuration both influence the fully absorbed cost, the enzyme production capacity is a more critical parameter, as shown in Figure 3. The break-even capacity (production throughput at which the fully absorbed cost equals the selling price) depends on the assumption in terms of market price; assuming a market price of $1,250 per g HRP, this capacity is slightly in excess of 5 kg HRP/year.

Under the base case assumptions, it is apparent that much of the cost lies in the capital equipment (see Figure 4), with finance costs accounting for 59% of the total cost of production, followed by fixed costs at 33%. Raw materials are a very small proportion of the total cost, being only $50/g HRP out of $1,279/g HRP (base case). The latter changes sharply when the adult plants are bought (not grown), with the raw material cost rising to $1,309/g HRP out of a total fully absorbed cost of $2,489/g HRP.

The greenhouse productivity, measured in both kg biomass/m^2/year and kg protein/m^2/year, is a critical parameter in the overall cost of the process. Actual values for the technology described in this analysis are 15.5 kg biomass/m^2/year and 3.7 kg protein/m^2/year. Progress towards an improvement in either value will have a big impact on the techno-economics; for instance if the yield of biomass can be improved from 5 g to 60g, thereby increasing the greenhouse productivity to 187 kg biomass/m^2/year, the fully absorbed cost will drop to $1,168. This target is not unrealistic considering the results of other laboratories with the infusion technology (4). Further recommendations

Figure 4. Relative and absolute proportions of the fully absorbed cost ($/g HRP) depend on the source of plants

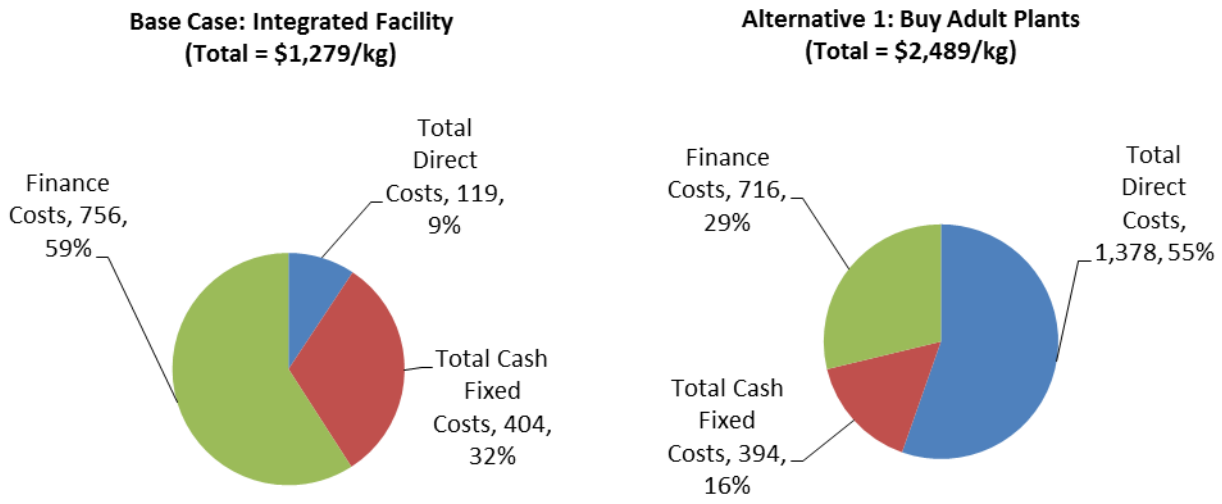
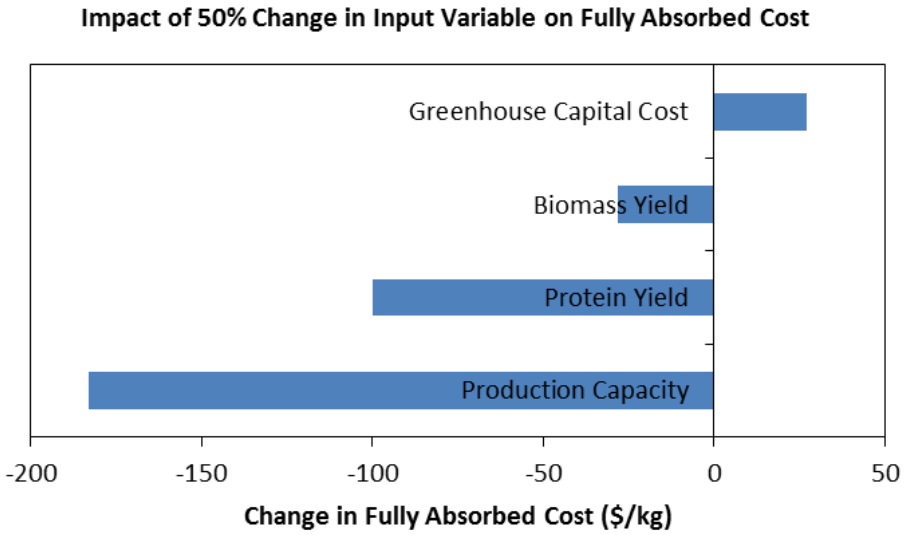


Figure 5. HRP sensitivity analysis



as to how the process economics can be improved are made in the following section covering the sensitivity analysis.

Sensitivity analysis

The results of a relative sensitivity analysis for HRP are shown in Figure 5. Assuming that proportional changes in the variables are of equal difficulty, the diagramme indicates that protein yield (AU/g biomass) should be the key focus of further research and development efforts. The unit cost of the greenhouse is of minor influence, but production capacity significantly changes the project viability with a 50% increase in capacity (from 5kg to 7.5 kg HRP per annum), resulting in a reduction in the FAC by $183/kg HRP. Although this capacity increase raises the capital cost, appreciable economies of scale are achieved at production volumes above 5 kg HRP per year.

Assuming that the two key parameters of the infiltration technology (protein yield and biomass productivity) could be improved through further optimisation on laboratory scale and that the production capacity can be increased, HRP production could become highly economical. For instance, with a doubling of biomass productivity to 30 kg/m^2/year, a doubling of protein concentration in the biomass to 480 mg/kg biomass, an increase in the protein yield from 54% to 63%, and a production volume of 20 kg HRP/year, the fully absorbed cost of production will be $611/g HRP vs. the selling price of $1,250/g. This scenario gives a NPV for the project of $39 million and an internal rate of return of 26%, making the project an attractive investment opportunity.

Comparison to Other Studies

As noted earlier, a limited number of studies have been published on the economics of protein production and recovery from plants (30, 3, 4, 13), and specifically on the use of transient expression for plant-based protein production (4). On the process economics of plant processes in general, Wilken and Nikolov (30) note the following:

- greenhouse costs are 20 to 25% and downstream costs are 65 to 90% of the total manufacturing costs (in this study the ratio was found to be 20% greenhouse and 80% downstream or purification costs)
- the production cost in a closed system (greenhouse) is 3 to 5 times larger than the equivalent cost in an open-field system
- the manufacturing cost for an open-field produced recombinant protein would be 30% - 50% lower than the cost of a bioreactor-produced protein under the assumption of equivalent downstream processing costs.

In other words, it can be inferred from the above that bioreactors are more economical than closed systems if the latter confer no additional advantage such as higher product quality, concentration or yield. Exactly how this may be achieved is outlined in a more detailed and specific study undertaken by Buyel and Fischer (4), who investigated the reproducible transient expression of a human monoclonal antibody (MAB) and a fluorescent protein in tobacco (*Nicotiana tabacum*) leaves. The study was designed to understand and hence optimise the factors governing variable levels of protein expression within each plant (leaf segments), between plants and between batches. A cost function was developed consisting of three components, namely the upstream costs for plant growth; the infiltration costs; and the downstream extraction and purification costs. The

function was then used to evaluate the economic outcome of different harvest schemes, and led to the conclusion that early harvesting of biomass reduced overall manufacturing costs by up to 30% even though total biomass was lower, a strategy described as "taking advantage of young, rapidly-growing tissues with high levels of protein synthesis" (4).

The Buyel and Fischer (4) model was applied to the data for HRP as used in this study and the results are shown in Table 5, from which it is noted that the Buyel model predicts a much higher cost per gram of product than obtained in this study ($25,500 per gram *vs*. $1,273 per gram), with the difference being mainly the consequence of higher levels of manufacturing quality associated with biopharmaceutical production (as opposed to industrial enzymes).

Conclusions

In summary, the technical progress of the infiltration technology has been considerable, and much progress has been made towards a novel commercial route for production of HRP. Although the product technology has yet to reach a feasible level, only minor optimisation is required to reach the economic targets.

The following specific observations have been noted.

- The infiltration technology is not competitive in our scenarios at scales of less than 5 kg HRP/year.
- Competing at lower capacity would be possible if the protein expression levels and/or the process yield of purified protein could be increased
- Similarly significant cost savings would be possible by increasing the plant yield

Table 5. Comparison of costs for MAB and HRP using Buyel model

	Units	MAB	HRP
Yield Plant Material	g bio/plant	60	5
Protein Expression	mg/kg bio	42	240
Number of Plants	plants/kg protein	571,429	1,543,210
Infiltration Cost	Euro/plant	0.93	0.93
Cost Growth Factor	Euro/plant	6.13	5.00
Unit Cost	Euro/g protein	10,981	19,099
	$/g protein	14,641	25,465

per tray or by reducing the plant growth cycle time; the key parameter for this aspect is the overall greenhouse productivity, measured as kg biomass/m^2/year (present and proposed target values are 15 and 200 kg biomass/m^2/year respectively).

- Some form of product differentiation relative to the existing product is desirable, such as a higher efficacy per unit of enzyme, higher stability, or lower cost. There is some evidence for both of the first two [14].
- Production volume changes the process economics considerably, as do protein yield and biomass productivity. Assuming that all the above targets can be met (market share, biomass productivity and protein yield), the fully absorbed cost of production will be $611/g HRP vs. the selling price of $1,250/g, giving a NPV for the project of $39 million, which makes the project an attractive investment opportunity.

Availability of supporting data

The data sets and methodology supporting the results of this article are included within the article, the supplementary material, and in the cited work (14).

Competing interests

The authors declare that they have no competing interests deriving from the subject matter of this work.

Authors' contributions

DW undertook the analyses and wrote the draft manuscript. EPR had overall responsibility for the work that provided the basis for the study, participated in its design

and helped to draft the manuscript. SMH performed the laboratory work and edited the manuscript. All authors read and approved the final manuscript.

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Supplementary Material

Explanation of SYC Input Assumptions

Discount Rate (% p.a.)	Usually close to a long term interest rate, the discount rate is the rate which is applied to calculate the net present value of a string of future cash flows. Discount factors will vary according to the country, the type of fund (private equity, merchant banking, etc.) and the credit rating of the borrower, with the range extending from 4% to 30% (and higher). For this analysis, a discount rate of 8% has been used.
Single Year Return on Investment (% p.a.)	The single year return on investment is the annualised minimum return on finance capital necessary to meet the investor's average cost of capital. The value is calculated assuming straight line depreciation of 10% per year, a discount rate of 8% and a working average cost of capital or 'average cost of debt' of 8%. The value is essentially the rate required to repay both the interest and the principal amount within the 10 year depreciation period.
Depreciation Rate (% p.a.)	The depreciation rate is the estimated annual decrease in the value of plant and equipment as a consequence of its use, and is assumed to be 10%. The latter value is standard in the process industries and is the average of short term depreciation (for computers and motor vehicles; usually 25% p.a.), depreciation on plant and equipment (10% p.a.), and buildings (usually 5% p.a.).

Overhead Costs (Direct Labour) (% of TCOE)	Overhead costs associated with direct labour are calculated by factoring the direct labour costs using an estimated overhead contribution for a 'standard' manufacturing company. The average value for such companies is 40% to 50% of direct labour, where the latter refers to labour directly involved in production, and covers items such as protective clothing, entertainment and information technology.
Overhead Costs (Indirect Labour) (% of TCOE)	Similarly, overhead costs associated with indirect labour are calculated by factoring the indirect labour costs using an estimated overhead contribution for a 'standard' manufacturing company. The average value for such companies is 90% to 100% of indirect labour, where the latter refers to labour not directly involved in production including management, clerical staff, and employees covering activities such as finance, human resources and logistics.
Working Capital Factor (% p.a.)	The cost of working capital is calculated on the basis of the total annual revenue, the cost of debt and a working capital factor, where the latter is an average factor for a manufacturing entity whose working capital requirements are the difference between debtors days and creditors days, plus stock days (typically 30, 45 and 14 respectively). Working capital factors vary from 5% to 25%; a value of 10% is used in this study.

Maintenance Factor (% p.a.)	The maintenance factor is the estimated annual cost of maintenance associated with an initial capital investment. The actual value can vary widely depending on the type of production facility; for this study a value of 5% p.a. (of the initial capital expenditure) has been used.
Distribution and Warehousing Factor (% p.a.)	The distribution and warehousing factor is used to calculate the annual costs to the manufacturing entity of these two activities. A value of 5% of total revenue is assumed, which is reasonable considering that distribution will take place though wholesale agents and stock holding kept at a minimal level.
Sales and Marketing Factor (% p.a.)	Similarly, sales and marketing in the SYC analysis is calculated by multiplying the total revenue by the sales and marketing factor, for which a value of 5% has been used in this study. The latter is considered reasonable on the basis of a minimal sales force with most product being shipped directly in bulk to the wholesalers or large scale assay kit manufacturers.
Royalty (% p.a.)	For this analysis, it has been assumed that a royalty of 5% will be payable to the inventors of the plant-based HRP production technology (University of Cape Town). The value is aligned with other royalty deals for a biological product whose market is the diagnostics industry.