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Guidelines and Cost Analysis for Catalyst Production in Biocatalytic Processes

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Abstract:

Biocatalysis is an emerging area of technology, and to date few reports have documented the economics of such processes. As it is a relatively new technology, many processes do not immediately fulfill the economic requirements for commercial operation. Hence, early-stage economic assessment could be a powerful tool to guide research and development activities in order to achieve commercial potential. This study discusses the cost contribution of the biocatalyst in processes that use isolated enzymes, immobilized enzymes, or whole cells to catalyze reactions leading to the production of chemicals. A methodology for rapidly estimating the production cost of the biocatalyst is presented, and examples of how the cost of the biocatalyst is affected by different parameters are given. In particular, it is seen that the fermentation yield in terms of final achievable cell concentration and expression level as well as the production scale are crucial for decreasing the total cost contribution of the biocatalyst. Moreover, it is clear that, based on initial process performance, the potential to reduce production costs by several orders of magnitude is possible. Guideline minimum productivities for a feasible process are suggested for different types of processes and products, based on typical values of biocatalyst and product costs. Such guidelines are dependent on the format of the biocatalyst (whole-cell, soluble enzyme, immobilized enzyme), as well as product market size and value. For example commodity chemicals require productivities in the range 2000−10000 kg product/kg immobilized enzyme, while pharmaceutical products only require productivities around 50−100 kg product/kg immobilized enzyme.

Introduction

Biocatalytic production holds great potential for clean and selective production processes and its application is steadily increasing in industry.[1−6] Furthermore, it is already established as a highly useful complement to conventional technologies for the production of optically pure chiral compounds in the pharmaceutical industry in particular.[1] Any new production process must pass a number of criteria to be successfully implemented. Safety, environmental, legal, economic, and throughput issues are all important aspects that need to be considered.[7] While biocatalytic processes are very competitive in terms of safety and environmental profile, one commonly discussed disadvantage is the cost of the catalyst.[8] Indeed, for lower value products, the industrial application of biocatalysis has thus far been limited, even though many potential processes have been suggested in the scientific literature.[5,9]

As has been stated elsewhere, it is frequently difficult to evaluate the cost of biocatalytic processes due to a lack of documented data on the factors contributing to the total cost.[9] Most available economic text books are focused on large-scale chemical manufacturing which makes it hard to draw parallels. Although biocatalytic processes as such can be very simple to operate, the development chain is generally more complex than for chemical processes.[2,9] It is therefore harder to estimate process cost (e.g., cost of the catalyst) and the cost of development, which in turn creates an uncertainty with respect to the risk of failure to meet the required cost of goods target. This frequently means processes may be discarded in error. For this reason there is a need for a better understanding of these costs so that the economic bottlenecks can be identified and addressed.[10]

Economic evaluation can be used as a decision-making tool to quantitatively estimate the expected profitability of a process, often alongside other criteria.[11] Cost estimates should be made throughout the early stages of a project even when comprehensive specifications (or other data) are not available.[12] However, methods for a full cost assessment are rather extensive and therefore take time to prepare. Consequently it is our contention that there is a need for methods that can simply and quickly assess not only if biocatalysis is a viable process option, but also identify the process bottlenecks. In this way guidance for research and development can be provided to give an understanding of when the process will achieve commercial success. This study presents a simplified approach for estimating the cost of different process scenarios, and ultimately the calculations can be used to evaluate process feasibility and identify bottlenecks. It should be emphasized that the results obtained should not be regarded as definitive values but as

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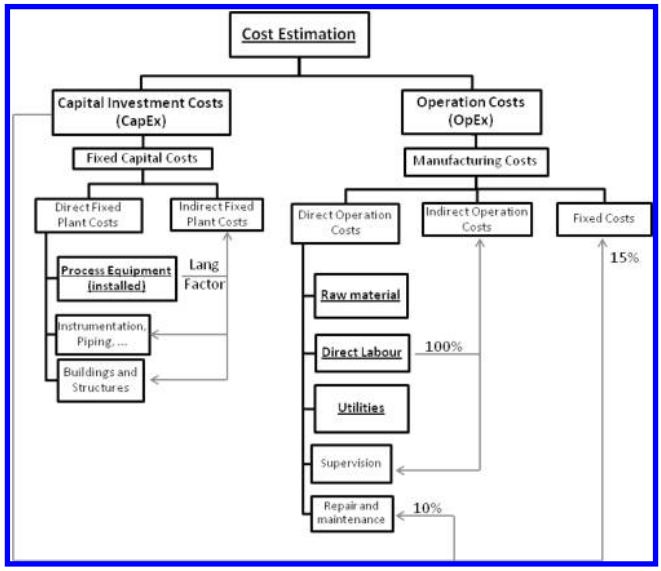

Figure 1. **Cost estimation categories and subcategories that are important for cost analysis. Underlined costs are calculated separately, while the other costs are estimated through the first ones, represented here with grey lines.**

guidelines that can serve as a starting point for other more detailed assessments.

The scope of this work is also to discuss and evaluate the cost of biocatalytic production processes with special emphasis on the cost contribution of the biocatalyst to the total production cost and the effect of scale-up, process and economic parameters. The dominating cost for different production processes and products is highly dependent upon the industry sector (i.e., pharmaceutical, fine, specialty or bulk chemical). This paper suggests minimum productivity requirements that need to be placed on the biocatalyst for processes in these different sectors (i.e., kg product/kg biocatalyst). It is well-known that process metrics such as product concentration (g/L) and space-time yield (g/L/h) are also very important for an economic evaluation but these are outside of the scope of this article.

Methodology

Cost estimation can be divided into two categories: capital investment (CapEx) and operation cost (OpEx), see Figure 1.

Capital Costs (CapEx). Fixed capital represents the capital necessary for the installed process equipment with all the accessories needed for the process start-up and operation.[11,13] In simpler approaches the calculation of CapEx is focused on the process itself, excluding site-wide auxiliaries, off-site and land-related items.[11−14] The foundation of a fixed capital estimate is equipment cost data. From this information the fixed capital investment can be calculated through the application of multipliers, such as the Lang factor.[13,15]

In order to obtain the total investment cost, the different parts of the direct CapEx (excluding equipment cost) and the indirect CapEx should be calculated separately (see Figure 1). However, in the early stages of process development the level of detail does not usually allow for an accurate and reliable calculation of these expenses. Hence, in order to obtain the total investment cost, the equipment cost is multiplied with a factor to cover the costs for all supporting equipment and services.[13] Detailed information concerning common factors used can be found in standard process design handbooks.[12,13]

The cost-capacity plot (six-tenths rule) is often applied when the effect of process scale is evaluated (see 1).

$$\text{Cost of equipment B} = \text{Cost of equipment A} \left(\frac{\text{Capacity of equipment B}}{\text{Capacity of equipment A}}\right)^{n} \quad (1)$$

where n may vary between 0.4 and 0.9, depending on the type of the equipment being costed, the operating conditions and the investigated range.[11,13]

To calculate the CapEx cost per production batch, the investment cost can be converted to an equivalent annual cost by multiplying the capital investment with an annuity factor, k (see eq 2).[11] The capital charge factor, i (or interest rate factor) is typically between 6 and 7% for the chemical industry but varies with, among other things, the risk of the project. The typical equipment economic lifetime, t, is 10 to 15 years.[13]

$$k = \frac{i}{1 - (1 + i)^{-t}} \quad (2)$$

Operating Cost (OpEx). The operating cost (OpEx) consists of direct, indirect and fixed costs. Direct operating costs includes the cost of raw materials, utilities, waste management and operating labor. Indirect and fixed operating costs can be calculated from direct labor cost and/or annual capital investment cost (see Figure 1).

The amount of raw material consumed is obtained from the process mass balances, and the cost of the most common chemicals can be obtained from the suppliers or by consulting trade journals (e.g., European Chemical News or Chemical Marketing Report).[11,13]

Utility requirements, including the cost of heating and energy for agitation, can be obtained from mass and energy balances and prices can be obtained from suppliers or purchasing agents. In fermentation processes, the dominating energy-consuming operations are often mixing and sterilization. The energy necessary for mixing can be calculated using rule-of-thumb values,[16] whereas the heat required for sterilization can be obtained using the heat capacity for water.

Although waste treatment is usually not part of the process design and cost model, waste disposal is an important process cost that should not be disregarded.[13,17] Typically wastewater treatment costs are 0.5−2 €/m^3 (depending on location), while nonhazardous solid waste disposal has a cost of around 25 €/ton.[17]

Finally, direct labor costs can be estimated from the process flowsheet based on typical labor needs for each unit operation[11] or by knowledge about labor requirements for the whole process. Labor rates can be obtained from the union contract,

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Table 1. **Summary of the considerations and source of information used in the economic model[a]**

cost	contribution to cost	consideration
CapEx	equipment cost	Matche Inc. (www.matche.com), process design software (ASPEN or SuperPro Designer)
	other capital investment costs	Lang factor:[15] 5.0 (typical for fluid processing units[18])
	annuity	From eq *2* For the base cases: $k = 0.142$, based on $i = 7\%$ and $t = 10$ years
	equipment scale-up	$n = 0.6$
OpEx	raw materials	market quotations, laboratory chemical suppliers
	utilities	0.1 €/kWh (European Energy Portal[19])
	waste handling	2 €/m[17]
	labor	30€/h (Eurostat[20])
	supervision cost and indirect opex	100% of the direct labor
	annual maintenance	10% of the annual capital investment cost
	fixed OpEx	15% of the annual capital investment cost

[a] k represents the annuity factor; i, the capital charge factor (or interest factor); t, the equipment economic lifetime.

from company labor relation supervision or from local statistical institutes (e.g., Eurostat, US Bureau of Labor Statistics).

Other operating costs can be calculated from direct labor costs or from annual capital investment. Supervision costs (direct operating costs) and indirect costs (including payroll overhead, quality control, royalties and plant overhead) normally correspond to 80 to 115% of the total direct labor costs. Annual maintenance (direct operating costs) including labor and material adds between 6 to 10% relative to the fixed capital investment.[11,13] Fixed costs are insensitive to the production scale and include depreciation, taxes, property rents, insurance, etc. corresponding to 12 to 17% the annual capital investment cost.[11,13]

Assumptions in Simplified Cost Estimation

As mentioned above, the aim of the present work is to develop a fast and accurate method for cost analysis. Since many data are not widely available, in particular when the process design is not fixed, assumptions have to be made. Table 1 summarizes the main considerations used to construct the proposed economic model.

When difficulty in obtaining raw material prices from the suppliers was experienced, the prices were estimated from laboratory chemical suppliers, by dividing the original price by 10 to 30 depending on the original package size. The uncertainty of this approach is high, but is still considered a good starting point for cost estimations. In the present case study the costs have been confirmed with industry.

The direct labor needs were determined through typical labor requirements and in discussion with industry. A value of 30€/h was assumed (Eurostat[20]) in order to calculate the cost associated with the direct labor. Labor needs are dependent on the plant scale and the degree of automation. However for processes within the same capacity range, the labor needs do not increase directly with process volume. Therefore, in this study it was assumed that labor needs did not increase with scale.

Evaluation of the costs in the preliminary design phases involves guesses and applications of rules-of thumb; therefore, the quality and accuracy of these estimations are dependent on the skill and experience of the engineer.[12] With the methodology applied in the presented study, its accuracy is considered to be on the order of ±30%. Regardless of the level of detail and complexity in an economic study and in the underlying project design, a certain degree of uncertainty will always remain.[13] This makes it is necessary to evaluate the effect of certain modifications to the original project on the total project cost.

Biocatalyst Production Costs

To determine the productivities required in a biocatalytic process to achieve a reasonable cost contribution of the biocatalyst, the manufacturing cost of the catalyst needs to be calculated. Here, these calculations have been divided into three main sections: fermentation, purification, and immobilization (see the first two sections of Figure 2). The influence of the costs on scale, accounting, and process parameters are also reported.

Fermentation. The production costs for a base case fed-batch fermentation of 10 m^3 were determined, assuming a final cell concentration of 50 g of CDW/L and 6.25 g of enzyme/L. Further, it was assumed that a single fermentation was run per week, and that the operation required a team of three full-time workers. Aspects of cGMP, such as validation and qualification protocols, and aseptic DSP processing have not been included into the calculations although these could be requirements in a final biotransformation step. The full details of the base case are given in Appendix I (Supporting Information).

Figure 3 shows the distribution of the costs and the production cost per kilogram of cells as well as per kilogram of enzyme (in the cell; nonpurified). It can be seen that in the base case the main cost drivers are equipment cost and labor costs, whereas utility costs are almost negligible. On the basis of our calculations, the production cost of one kilogram of cells is €67, corresponding to a cost per kilogram of enzyme (within the cell) of just over €500.

By analyzing the distribution of the different costs versus production volume it can be seen that the impact of the different costs varies greatly with scale. For instance at small scales (<10 m^3) the greatest cost contribution comes from labor and equipment costs, whereas at a larger scale (>50 m^3) the impact of labor is small, and the cost of the raw material becomes

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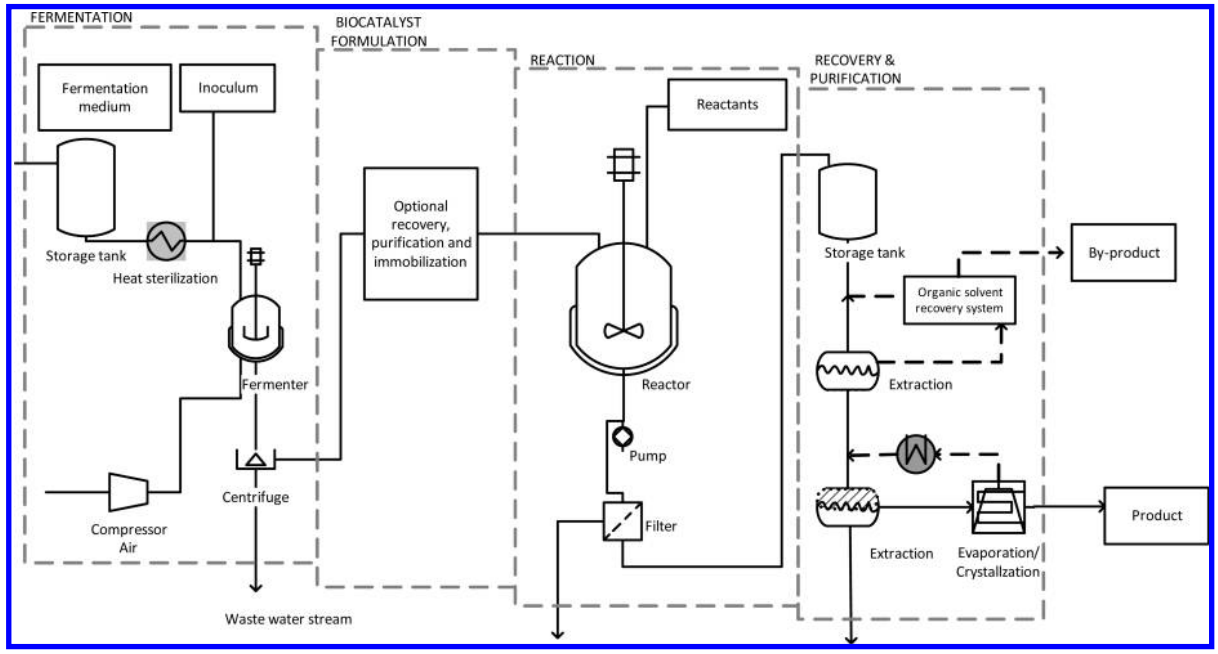

Figure 2. **Example of a theoretical biocatalytic process, including biocatalyst production (fermentation and biocatalyst formulation), biocatalysis (reaction), and downstream processing (recovery and purification). Note: The biocatalyst is normally produced independently from the reaction step and then stored until use.**

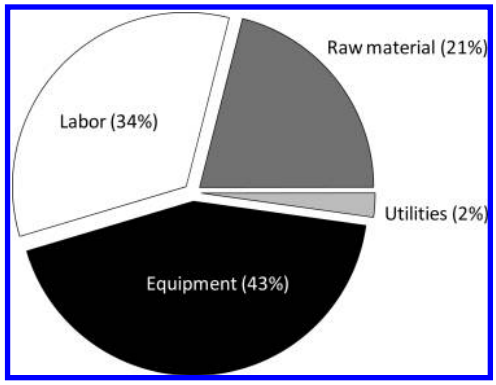

Figure 3. **Distribution of costs in the base case fermentation.**

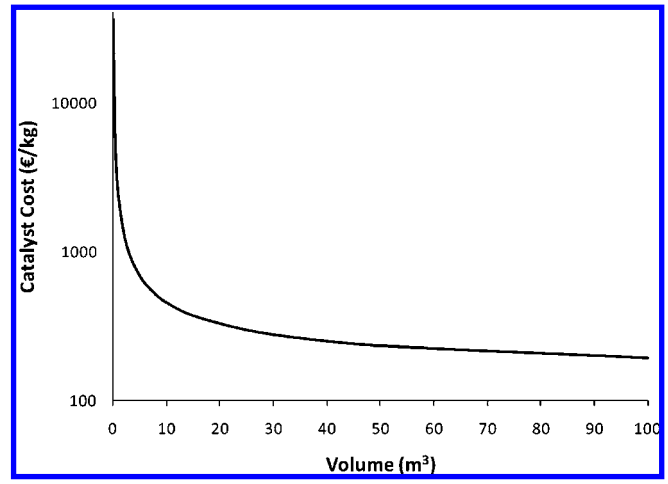

Figure 4. **Effect of scale-up in total production cost.**

dominant. The obtained trend is in accordance with other published reports.[21]

Sensitivity Analysis. Emphasizing the fact that the cost of the biocatalyst will depend on many variables, a sensitivity analysis was carried out to determine and visualize the impact of different process parameters on the biocatalyst production cost. The analysis was carried out by varying one or more input parameters in the economic model to see the effect on the costs. All figures in the sensitivity analysis section are plotted as a cost factor relative to the base case in order that these can be combined to represent a specific case.

Effect of Scale. As discussed previously, one of the most important parameters in the process is the production volume. By varying the production volume in the model, the impact on the cost per kilogram of enzyme was plotted (Figure 4). It can be seen that the production costs decrease rapidly when increasing the scale from 100 L to multiple cubic meter scale and that the cost can be more than halved when increasing the scale from 10 m^3 (the base case) to 100 m^3. However, at very high working volumes momentum, mass and gas transfer limitations are encountered in aerated fermentors. Because of this, the graph will not follow the mathematical model anymore and the points to the extreme right are speculative. On the other hand, at larger production volumes relatively lower cost of raw materials could be expected which would also reduce the total cost of the catalyst.

The general picture that cost of enzyme is dependent on scale means that the market size for a given application is of paramount importance to the selling price of the enzyme.

Effect of Equipment Cost and Utilization. As is clear from Figures 3 and 4, the equipment costs are an important contribution to the cost at practically all scales within the investigated range. A sensitivity analysis was performed directed at the assumptions controlling the equipment costs, i.e. equipment purchase cost (see eq 1), interest rate, economic lifetime of equipment (see eq 2), and equipment utilization. As can be seen from Figure 5, these assumptions also have a significant impact on the total production cost. Most notably the utilization of the equipment (i.e., the number of batches that can be run per year)

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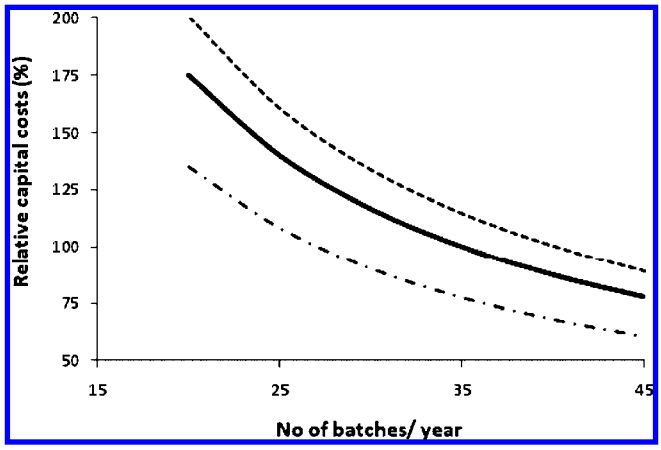

Figure 5. **Impact of equipment purchase costs, utilization, economic lifetime (depreciation), and interest rate on the cost of production in the base case $n = 10$ y, $i = 15\%$; $n = 10$ y, $i = 7\%$; and $n = 15$ y, $i = 7\%$.**

has a great impact on the cost of the enzyme, emphasizing the importance of equipment efficiency (in terms of occupancy). Hence, one can easily understand that ideally the equipment occupation time should be maximized. In our base case, calculating the full fermentation time including setup, harvesting, and cleaning is assumed to be one full working week, although the fermentation time is only 48 h. This results in a low over all productive occupancy (<30%). Indeed, for larger plant facilities the equipment can often be shared among different process lines and can therefore be used more efficiently, thereby reducing the cost of using the equipment. However, one of the main reasons for lengthy downtimes is to reduce the risk of cross contamination. This is a critical issue that needs to be properly addressed, especially in the pharma business as a consequence of GMP regulations.

It can also be seen from Figure 5 that the assumptions regarding interest rate and equipment lifetime has an effect of ~±20% on equipment costs, when varied between 7−15% interest rate and 10−15 years of plant lifetime.

Effect of Fermentation Yield. In the last part of the sensitivity analysis, the yield of enzyme in the process was varied. In an intracellular production system (e.g., *Escherichia coli*) it is possible to obtain yields up to ~15 g/L, after which the system is limited by the cell density (~100 g CDW/L) and internal protein composition (~30% of protein composition[21−23]). Higher levels also run the risk that the protein is expressed as an inclusion body. For an extracellular enzyme production system (e.g., *Pichia pastoris*) higher enzyme levels can be reached. In this study, an upper limit of 25 g/L was assumed, but even higher titers have been reported. In the base case a yield of 6.25 g enzyme/L was assumed, which would represent a somewhat optimized and reasonably successful production system.

As can be seen from Figure 6, the yield has a dramatic effect on the costs of the enzyme, particularly in combination with changes in production scale. This means that enzyme cost could easily vary between tens of thousands of euros per kilogram

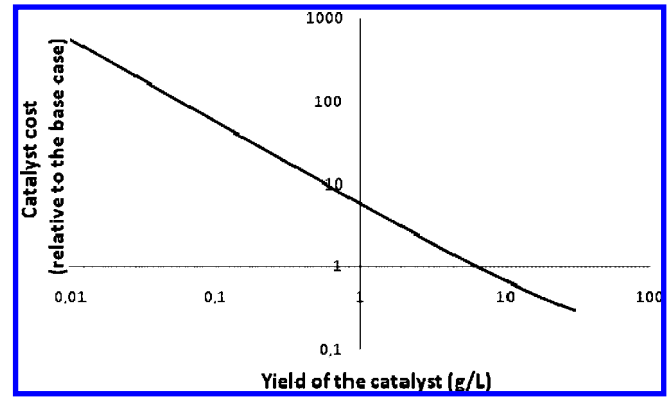

Figure 6. **Sensitivity analysis. Impact of enzyme yield on costs relative to the base case.**

down to less than 200 euros per kilogram. For instance, if the yield of enzyme is 10 mg/L instead of the base case 6.25 g/L, the cost per kilogram of enzyme is increased 500-fold. On the other hand, if the yield can be increased to 15−25 g/L the cost of the enzyme can be cut to a half or a third of base case costs.

A reasonable assumption on enzyme production cost (excluding development costs) for a developed production system on an industrial scale could therefore be between 250−1000 €/kg for an unpurified enzyme and similarly for whole-cells, 35−100 €/kg. However, some types of enzymes are more expensive to produce than others. For instance peroxidases, which require a heme group to be incorporated in the active site to be able to catalyse oxidations, are difficult to produce with high titres of active enzyme and consequently become much more expensive than the base case in this study.[24,25]

Catalyst Formulation. An important stage in the development of a biocatalytic process is to choose the form of catalyst to be used. The active enzyme can be kept inside the host cell (i.e., whole-cell catalysis), or it can be used as an isolated enzyme. If the isolated enzyme is to be used, it is also important to determine to what extent the enzyme needs to be purified, since this greatly influences the production cost.[26] The choice of catalyst form affects the process in a number of ways: the stability of the enzyme, the possibility for recycling of cofactors, selectivity, mass-transfer, etc.[8] According to an analysis performed by Straathof and co-workers,[5] about 60% of the reported industrial biocatalytic reactions use whole-cells (in either free or immobilized form) as catalysts, with the remainder using either soluble or immobilized enzymes.

As will be seen in the later sections of this article, the low allowed-cost contribution for bulk and commodity chemical production processes necessitates a high catalyst productivity, i.e. a large amount of product per kilogram of catalyst. One way of limiting the enzyme consumption would be to use very little enzyme in each reaction. However, since it is normally desirable to keep the reaction volume as low as possible, the demand for high space-time yield typically translates into a need to reuse the enzyme.[27] This means that a method for separating the enzyme from the reaction mixture is required, either by retaining the enzyme in the reactor or by separating it from the

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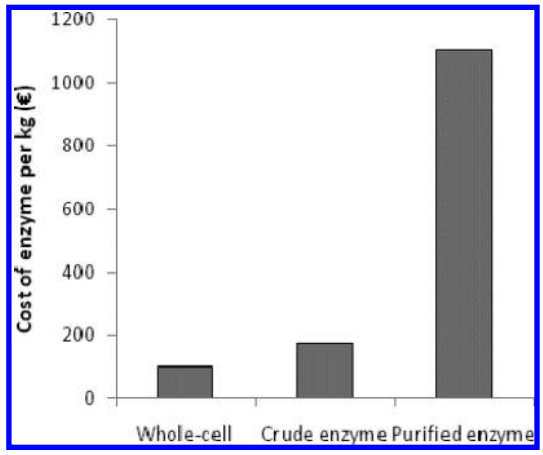

Figure 7. **Cost of purification of enzyme catalyst.**

outgoing product stream. One useful way of achieving this is by immobilizing the enzyme.[28−30] An added advantage of immobilization is that it allows enzymes to operate in systems where they are not usually soluble, such as in organic solvents.

It is important to recognize that the intrinsic enzyme properties, including activity and stability, can be quite different in an immobilized preparation compared to the soluble form.[27] Since these catalysts are heterogeneous, they will also be subject to mass transfer limitations that can reduce the overall activity and potentially selectivity of the enzyme.[31] These issues have not been considered in the present study, but are mentioned here to illustrate that modifications to a biocatalyst in order to suit a given application require additional time and cost for development and implementation.

Recovery and Purification. For whole-cell biocatalysts, application in the reactor may proceed directly (normally after centrifugation or filtration to replace the fermentation medium and/or adjust concentration). For an enzymatic catalyst (whether used in soluble or immobilized form) the costs for recovery and purification need to be estimated. In order to illustrate this, the cost of three different biocatalyst formulations was analyzed: whole-cell, crude enzyme, or purified enzyme, based on a process for manufacturing β-galactosidase in the process simulation software, SuperPro Designer.[32] The cost of the product was calculated after the different recovery and purification steps. The whole-cell biocatalyst was recovered by microfiltration; to obtain crude enzyme the cells were run through a homogenizer, centrifuged to remove cell debris, and finally submitted to ultrafiltration. Partially purified enzyme was prepared by additionally running ion-exchange and gel filtration chromatography as well as two additional ultrafiltration steps. As can be seen from Figure 7 the added cost in each step is significant. The preparation of crude enzyme from whole cells adds to the specific cost of the enzyme by a factor of almost 2. Needless to say, this value could be significantly reduced by developing an extracellular production scheme. Furthermore,

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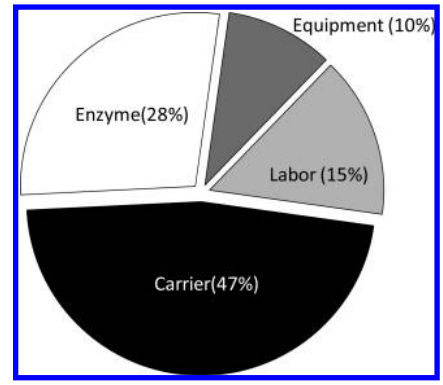

Figure 8. **Cost distribution for the base case.**

purification by chromatography adds almost an order of magnitude to the cost where the major cost contribution comes from the consumables such as the resin material. From this analysis it follows that it is very important to weigh the cost of purification against the added value of higher enzyme purity. The general rule-of-thumb is that the crudest possible form of enzyme acceptable, to maintain product quality, should be used.[10]

Immobilization of Biocatalyst. In this work immobilization by adsorption has been used as an example to illustrate the principle of costing an enzyme immobilization process, based on the parameters outlined in Supporting Information, Appendix 2. This particular immobilization procedure involves preparation of the enzyme solution and adsorption of the enzyme onto a carrier from solution, followed by filtration and drying of the preparation for storage and use.[28,31]

Figure 8 shows the distribution of costs in the base case for adsorption immobilization; e.g. raw material accounts for 75% of the costs of the catalyst. With these conditions, the immobilization increases the specific enzyme cost by a factor of 4, from 500 €/kg to 2000 €/kg, although the per kilogram cost of the catalyst is of course lower, 100 €/kg of immobilized enzyme.

Immobilization Sensitivities. The calculated 4-fold increase in enzyme cost upon immobilization for the base-case is linked to the assumptions listed in Appendix 2 (Supporting Information). Figure 9 shows the effect of variations in enzyme and material (e.g., carrier) cost, as well as labor intensity and batch size, on the final biocatalyst cost. The impact of the cost of the carrier and the labor intensity is directly proportional to the cost contribution of each in the base case. Hence, greater accuracy is required in the estimation of the material cost (a similar argument can be made for the enzyme loading on the catalyst).

The impact of production scale (batch size) is more complicated. In the base case, material costs constitute 75% of the total costs, and the final catalyst cost is thus relatively robust with respect to the production volume; reducing the batch size by 5% only increases the cost increase by 1%. Similar effects are obtained with a variation in equipment utilization (data not shown).

The relative added cost of the immobilization procedure is highly dependent on the enzyme cost, or rather the cost of the enzyme relative to the cost of added materials, labor, and equipment. For the base case, this means that the relative cost increase of the enzyme would be considerably lower for a more

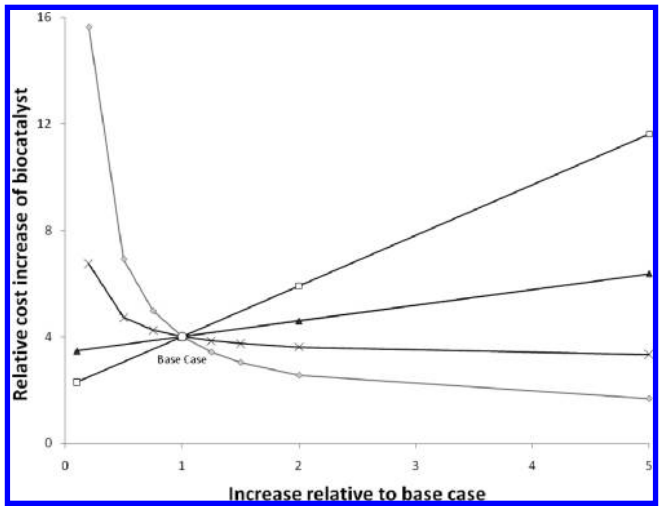

Figure 9. **Variation in relative cost increase for an immobilized enzyme with changes in cost of enzyme (◇) and other materials (□), labor intensity (▲), and batch size (×), relative to the base case.**

expensive enzyme. Although this indicates that the cost of the immobilization procedure is more critical for less expensive enzymes, the final choice of catalyst must be based on a balance between both cost and performance in the intended application.

Figure 9 shows the sensitivity of the biocatalyst cost when the key parameters are varied (cost of the free enzyme, cost of the carrier, labor intensity, and batch size). The cost variation and the slope of the variation are highly related with the cost distribution (see Figure 8), meaning that a variation in the carrier cost will strongly affect the cost of the immobilized catalyst, since it accounts for the biggest share of the total cost in this case.

By varying the different production parameters, i.e. enzyme cost, carrier cost, production scale, etc., it was found that a range of cost for the immobilized enzyme (enzyme adsorbed on resin) of 100−1000 €/kg is a reasonable assumption for further calculations.

Application of Economic Analysis

The role of economic analysis as illustrated by the above examples is three-fold. First, it can provide guidelines for targets which need to be achieved, such as the production yield (kg product/kg catalyst). Second, it can identify key bottlenecks in a process (such as the biocatalyst production or downstream process) by identifying process performance sensitivities (such as production yield, fermentation yield, and recovery yield) to operating variables and process design. Finally, the combination of these analyses leads to a strategy for process development and improvement by introducing new targets, such as increase of production yield by protein engineering,[33] improving expression system,[34] or improving product recovery steps by introducing *in situ* product removal.[35]

Guidelines for Biocatalyst Productivity Targets. As can be seen in the analysis above, biocatalysts are relatively expensive compared to other raw materials in a process in terms of cost per kilogram. However, the price of a catalyst does not mean much in itself. The important question is how much the catalyst contributes to the cost of the product compared to the added value of using biocatalysis over other production methods. The added value could be achieved through higher yield, milder reaction conditions, higher product purity, fewer reaction or purification steps, improved safety, reduced emissions to the environment, or the manufacture of a unique product.[7] In the following section the productivity requirements in terms of kilogram of product produced per kilogram of biocatalyst is calculated for different types of chemicals (bulk to pharmaceuticals) when using either whole-cell, free enzyme, or immobilized enzyme as the biocatalyst. Finally these requirements have been summarized in Table 3.

The productivity requirements (in terms of product produced per kg of biocatalyst) are related to the allowable cost contribution of the biocatalyst and the cost of the biocatalyst by the following equation:

$$\text{productivity target} = \frac{\text{biocatalyst cost}}{\text{allowable cost contribution}}$$

Different types of chemicals generally put different requirements on the allowable cost contribution of the catalyst. A high volume bulk or commodity chemical, typically priced in the range of 1 €/kg,[36] could be assumed to allow the enzyme to contribute about 5% of the selling costs, i.e. around 0.05 €/kg. For specialty or performance chemicals, such as cosmetic ingredients and food supplements, prices are somewhat higher. If a selling cost from 5 €/kg is assumed, the allowable cost of the biocatalyst could be around 0.25 €/kg.[30]

In the fine and pharmaceutical chemical segment product values are considerably higher, up to hundreds of euros per kilogram. In the framework of the present study it was assumed 15 €/kg for fine chemicals (pharmaceutical intermediates) and 100 €/kg for finished small molecules for use as pharmaceuticals. In such cases the higher-value, smaller-market, and increased process complexity would allow for a higher cost contribution of the biocatalyst. For example, if 10% is assumed, then the allowable contribution for fine chemicals is 1.5 €/kg, and for pharmaceutical intermediates, 10 €/kg.

In the case study presented here the ranges of production costs for the different forms of the biocatalyst were found to be 35−350 €/kg DCW for the whole-cell, 250−2500 €/kg for the crude isolated enzyme, and finally 100−1000 €/kg for the immobilized biocatalyst. However, the different market volumes of bulk and pharmaceutical products mean that development costs need to be shared on a widely different volume of biocatalyst and also that the production volume of the biocatalyst will be different (which also affects the biocatalyst production costs).

As can be seen from the results presented in Table 3, the required productivity targets range over several orders of magnitude, depending on the type of catalyst and product. The

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Table 3. **Required productivities for different types of processes and products, based on typical values of biocatalyst and product cost**

	typical product cost (€/kg)	allowable cost contribution of enzyme (€/kg)	biocatalyst cost	range of required productivity
pharma	>100	10	whole-cell: 100−350 €/kg DCW free enzyme: 1000−2500 €/kg enzyme immobilized enzyme: 500−1000 €/kg biocatalyst	10−35 kg product/kg dry cell weight 100−250 kg product/kg free enzyme 50−100 kg product/kg immobilized enzyme
fine chemical	>15	1.5		70−230 kg product/kg dry cell weight 670−1700 kg product/kg free enzyme 330−670 kg product/kg immobilized enzyme
specialty chemical	5	0.25	whole-cell: 35−100 €/kg DCW free enzyme: 250−1000 €/kg enzyme immobilized enzyme: 100−500 €/kg biocatalyst	140−400 kg product/kg dry cell weight 1000−4000 kg product/kg free enzyme 400−2000 kg product/kg immobilized enzyme
bulk	1	0.05		700−2000 kg product/kg dry cell weight 5000−20000 kg product/kg free enzyme 2000−10000[a] kg product/kg immobilized enzyme

[a] Productivity values similar to this have been reported in a number of well-documented commercial processes such as the production of high fructose corn syrup with glucose isomerase[37] and biocatalytic acrylamide synthesis.[38]

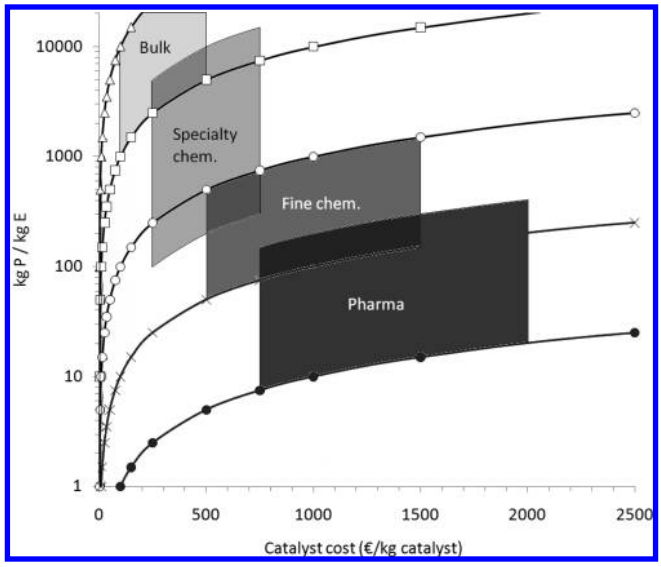

Figure 10. **Effect of biocatalyst cost and allowable cost contribution on the requirements for biocatalyst productivity in terms of kilogram of product per kilogram of biocatalyst used for production of bulk, fuel, or specialty chemicals using immobilized enzymes. Allowable cost contribution of 0.01 €/kg △, 0.1 €/kg □, 1 €/kg ○, 10 €/kg ×, 100 €/kg ●.**

large difference in productivity requirements between whole-cell and crude enzyme is striking, although this is mainly due to the difference in enzyme concentration of the two preparations. Further, as is clear from the sensitivity analysis on biocatalyst cost (discussed earlier), the cost of the biocatalyst could be orders of magnitude higher for a nonoptimized process, and in addition the added value from introducing a biocatalyst can vary very much between different processes. To illustrate this, Figure 10 shows the correlation between allowable cost contribution of the enzyme, cost of the biocatalyst, and required productivity for different types of processes, using as an example the use of an immobilized biocatalyst.

The top left box in Figure 10, represents bulk processes with an allowed cost contribution of from 1 cent per kilogram to 10 cents per kilogram and the cost of the biocatalyst in the low range (100−500 €/kg) because of the large production volumes. Slightly overlapping is the box representing specialty chemicals, which have a quite broad range of allowable cost contribution due to the many different types of chemicals in this group. The cost of the biocatalyst will probably be somewhat higher than that for bulk processes. Further down to the right are the boxes for pharmaceutical intermediates and small-molecule pharmaceutical products with allowable cost contributions of the biocatalyst much higher than that for bulk and specialty chemicals, but at the same time, higher costs for the biocatalyst. On one hand, it can be seen that for low-value bulk chemical processes (such as for biofuel) it is likely that a productivity of more than 10000 kilograms per kilogram of catalyst will be required. Even productivities that cannot realistically be achieved using biocatalysts could be required if the biocatalyst cannot be efficiently produced or if the added value to the process is very low. On the other hand, a higher-priced bulk chemical with a high margin for biocatalyst cost could allow for productivities down to about 1000 kilograms of product per kilogram of biocatalyst if a low-cost catalyst could be manufactured. For specialty chemical processes the range could be even larger, from several thousand down to less than a hundred kilograms of product per kilogram of biocatalyst. Finally, for pharmaceutical intermediates and small-molecule pharmaceutical products the required productivities are lower and lie in the range of 50−1000 for pharmaceutical intermediates and 5−100 for small-molecule pharmaceutical products.

In conclusion, more expensive products can carry a higher catalyst cost—suggesting lower productivity requirements—but these products normally have a smaller market size. Consequently, the catalyst production cost will be higher. The definitive productivity required to ensure that a process is economically viable needs to be evaluated on a case by case

basis. Nevertheless, the values suggested in Table 3 will be a useful starting point for setting development targets in different process sectors.

Towards Process Improvement. As previously mentioned, the economic analysis of a biocatalytic process is a useful tool for process improvement. Productivity targets can be set as a basis for improvement. What to improve is set by identifying the process bottlenecks (or the parts of the process preventing the process from being economic).

For example, sensitivity analysis on biocatalyst yield (see Figure 6) can be used to set the development targets for the R&D department. Subsequently, on the basis of the requirements of the specific process, a strategic decision needs to be made if the required targets can be met with reasonable development effort in terms of time and money. In the example of biocatalyst production, for a nonoptimized or wild-type expression system, quite low yields are probable; a starting point in the milligram of enzyme per liter range could be considered as reasonable. On the other hand, optimized production systems can (and must) achieve much higher yields (using genetically engineered microorganisms[21,22]). However, these high yields require highly optimized production protocols and expression systems, which normally take many weeks or months to develop.[39] This comes at a significant cost that in the end also needs to be carried by the product. However, as these costs are very difficult to estimate and the added cost per kilogram of product depends also on the sales volume, this has not been included in the current model.

Concluding Remarks

Process cost estimation is extremely useful, both in production as well as in R&D, to guide activities directed at developing, implementing, and improving processes. Much useful information can be obtained about the drivers and bottlenecks preventing the immediate implementation of an effective and economic process, even at an early stage of development (where the uncertainties are considerable). Process cost estimation can therefore be very useful as a decision-making tool.

The study we report here shows that many factors work together in determining the cost of the biocatalyst and that the range of cost is therefore rather wide (from hundreds of Euros per kilogram of enzyme up to several thousands of Euros per kilogram). In the first step of the production (fermentation), the enzyme titer is crucial; a product yield in the gram per liter range is required to avoid excessive costs. This means that almost without exception, significant effort must be put into developing the fermentation process before it is ready to be used industrially. It also means that analyzing the production cost at an early stage of process development will overestimate the cost of the mature process. Moreover, the scale of production greatly influences the production cost, especially at volumes less than ∼100 kg per batch (∼20 m^3). Finally, any purification steps might also increase the production costs within an order of magnitude.

As with any new technology, a cost/benefit analysis has to be performed to weigh the added cost of the biocatalyst against the value of the process improvements. This study has shown that, for low-value, large-volume products, the required biocatalyst productivity is in the range of 2000−10000 kg/kg immobilized enzyme. For higher-value products, the required productivity is, of course, lower; nevertheless, even for high-priced fine chemical compounds there are high productivity requirements, ∼50−1000 kg/kg, due to the lower production volumes and thus higher cost of the biocatalyst. As proven by the number of industrially implemented biocatalytic processes, these target productivities can be reached, but low-volume specialized catalysts can only be applied to processes where they can contribute to the process via significant improvement or achievement of very high productivity. Correct assessment (as well as consistent documentation) of catalyst productivity is therefore essential to determine the viability of a biocatalytic process, and is something that should be emphasized in any study of biocatalysis.

In a biocatalytic process, directed development of the catalyst specifically for the reaction of interest is frequently required. However, some industries (such as the pharmaceutical industry) cannot afford time-consuming research on protein development, and the possibility for process development is limited. Hence, the development of industrial biocatalysis is dependent on the availability and use of already developed biocatalysts and ultimately the enlargement of technological platforms.

In many ways biocatalytic processes can still be considered a technology under development (which has not yet reached its full potential), and much work remains before platform technologies are available, allowing quick and consistent development of efficient and cost-effective biocatalytic processes. Furthermore, academia and R&D departments in industry should join efforts aimed at the development of given technological platforms embracing fermentation and biocatalyst and process development for particular reaction types. Such platforms should also be a source of information concerning the development of fermentation and catalyst production (as the pluGbug developed and commercialized by DSM), development of the catalyst (as the effort put in by Novozymes on its lipase, Novozyme 435), and process development (such as technologies for *in situ* substrate supply and product removal[3,24,35]). A range of reactions should be considered to extend the currently available technologies.

The establishment of a suitable platform might guide the development of different products and processes, leading to a common effort towards the improvement and wider application of biocatalysis in industry.

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Supporting Information Available

Appendices 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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