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Opinion

TRYing to evaluate production costs in microbial biotechnology

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Microbial fermentations offer the opportunity to produce a wide range of chemicals in a sustainable fashion, but it is important to carefully evaluate the production costs. This can be done on the basis of evaluation of the titer, rate, and yield (TRY) of the fermentation process. Here we describe how the three TRY metrics impact the technoeconomics of a microbial fermentation process, and we illustrate the use of these for evaluation of different processes in the production of two commodity chemicals, 1,3-propanediol (PDO) and ethanol, as well as for the fine chemical penicillin. On the basis of our discussions, we provide some recommendations on how the TRY metrics should be reported when new processes are described.

Highlights

Microbial fermentations are widely used for the production of chemicals used as pharmaceuticals, food ingredients, materials, solvents, and biofuels.

Technoeconomic analysis of a given fermentation process is important to perform before scaling the process to levels that enable commercial production.

Titer, rate, and yield (TRY) of the fermentation process are key metrics that are used for technoeconomic analysis.

TRY metrics have different impacts on the technoeconomic analysis, and it is important to be aware of these differences.

Industrial biotechnology and impact of metabolic engineering

In industrial biotechnology, microbial fermentation is used to produce a wide variety of chemicals used in agriculture, household care products, cosmetics, and the food and pharmaceutical industry and as biofuels. Traditional products include organic acids (lactate, citrate), antibiotics, amino acids used as feed additives, vitamins used for both humans and livestock, enzymes used in detergents and a variety of industrial processes, and ethanol used as a biofuel. In recent years, microbial fermentation processes have, however, also been developed to produce **commodity chemicals** (see Glossary) used to produce materials as well as to produce fine chemicals used as ingredients in food and cosmetics (Box 1). A key driver for this development has been our ability to engineer microbial cells to have a tailored metabolic network that is well suited to produce one specific product, generally referred to as **metabolic engineering** [1,2]. Over the past 20 years, there has been a tremendous advance in the field of metabolic engineering [3], and the literature reports hundreds of academic studies on the production of different chemicals that can have potential use in the market. However, for these academic projects to advance, it is important to scale the process and ensure that the process can meet certain technoeconomic targets. Here the **cost of goods sold (COGS)** is a key parameter for evaluating a new process, as this will define if the product can compete in the market. This holds when an alternative production method is proposed for a chemical that already has an established market, as well as when a new chemical is made that has to be positioned in the market. COGS is basically determined by the following cost factors: (i) raw material costs, (ii) operational costs, (iii) depreciation of the production facility, and (iv) depreciation of the research and development costs. The latter can greatly differ, depending on the product. For example, the development costs of novel pharmaceuticals are often higher than those of commodity chemicals because of costly clinical trials and registration fees. As we discussed recently [4], the research and development costs for engineering a new strain have decreased significantly in the past 10 years, and they therefore today account for only a small fraction of the costs of developing a new process. Furthermore, even though it can be costly to scale a new process, this normally results in the production of some amounts of products that can be sold or used to develop the market, and, in the overall

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Box 1. Commodity, specialty, and fine chemicals

Commodity chemicals are a group of chemicals that are produced in large volumes as they address large and global markets, typically to produce plastic, **renewable chemicals**, and other materials. Examples are (i) acrylic acid used for the production of acrylates that can be used as superabsorbent materials in, for example, diapers; (ii) caprolactam used for the production of nylon 6; (iii) ethylene used for the production of polyethylene and other plastics; and (iv) propylene for the production of polypropylene. Ethylene and propylene are the two chemicals produced in the largest volume, with more than 150 million tons of ethylene and more than 80 million tons of propylene being produced annually. There is only one fermentation product that comes close to this kind of volume, namely ethanol, which has an annual production exceeding 100 million tons. Most commodity chemicals are sold for around 1–3 USD/kg.

Specialty chemicals are a group of chemicals that can serve specific functions in an application, for example, as adhesives, agrochemicals, detergents, cosmetic ingredients, flavors and other food additives, fragrances, and surfactants. The market volume of specialty chemicals is much lower than that of commodity chemicals, but they also typically have a higher price, that is >5 USD/kg. Fine chemicals are a subgroup of specialty chemicals and are characterized by being complex, single, and pure chemical substances and are typically produced in limited quantities.

cost analysis, these costs are therefore also often marginal. Therefore, the three first-mentioned cost factors are important to evaluate, and these are determined by three key metrics for a fermentation process: **titer**, **rate**, and **yield**, often called 'TRY.' Here we describe the concepts of the TRY parameters and illustrate how they impact the economics of various processes. Throughout we refer to different fermentation modes described in Box 2, but we do not discuss the pros and cons of these different fermentation modes.

Importance of the TRY metrics for technoeconomic analysis

The three TRY metrics impact technoeconomic analysis differently, as discussed in the following text. For a mathematical definition of the metrics, see Box 3.

(i) Titer (product amount per volume, c_P) represents the final concentration of the product in the fermentation process. This is the easiest metric to measure and is therefore also the one used most widely to evaluate the performance of a microbial fermentation process. The titer is determined by the volumetric productivity, that is, how much product is formed per unit of fermentation volume (g product/l reactor/h) and the fermentation time (Figure 1A). The volumetric productivity is a function of the biomass-specific productivity of the cell factory (g product/g biomass/h), that is, how much product is produced per unit of biomass per unit of time, and the biomass concentration (g biomass/l reactor), and as these two variables change during a typical fermentation process, the volumetric productivity varies throughout the process,

Box 2. Different cultivation modes

Bioreactors are generally run in three different modes or combinations of them: batch, continuous, and fed-batch [17]. Batch cultivations have no flow in or out of the reactor, and the volume is constant. However, this is a theoretical assumption and does not reflect reality. In practice, the volume will change, for example, by the addition of acid or base to adjust the pH or through evaporation (which can be reduced through the installation of a condensator). The TRY metrics for batch cultivation are displayed in Figure 1 in the main text.

Continuous cultivations have a constant flow in and out of the reactor while the volume is constant. This allows the constant supply of fresh media to allow for constant growth over long fermentation times. A batch phase for the generation of biomass usually precludes the continuous phase. After the switch to the continuous phase, an oscillating behavior of many process parameters can be observed. After this, the culture reaches a balance in which all parameters are stable (see Figure 3 in the main text). Although this can lead to high volume-specific productivity, it is limited by the genetic stability of the engineered strains and the risk of contamination. It therefore finds less application in industrial processes.

Fed-batch cultivations constantly feed fresh media into the bioreactor in an exponential manner, whereas there is no flow out of the reactor (except for evaporation). This allows the cultivation to reach high cell densities and titers and is the preferred industrial cultivation mode. However, because of the high cell density, the oxygen transfer rate (or cooling) becomes the growth-limiting step at which point the fed volume is kept constant. The oxygen limitation reduces the growth rate of the cells and often increases the overall maintenance costs of the cell. This in turn can result in lower production rates.

Glossary

Chemostat: a bioreactor cultivation in which fresh medium is constantly added while the culture volume is kept constant, resulting in limiting conditions (usually carbon limiting). In chemostat cultivation, cells reach a steady state, and the dilution rate (D =F/V) equals the growth rate of the organisms in ideally mixed systems ($x = x_{out}$).
Commodity chemicals: chemicals produced in very large quantities that are used by the chemical industry as building blocks for the production of solvents and materials.
Cost of goods sold (COGS): specifies the unit cost the product can be sold at with profit. For commodity products that are produced and sold in very large volumes, the profit per unit product is normally low, and COGS is therefore close to the cost of producing the product.
Metabolic engineering: targeted genetic modifications of cell factories with the objective of producing novel chemicals and/or improving the product yield.
Rate: the rate of product formation. There are two types of rates: the biomass-specific rate, which is the rate of product formation per unit of biomass, and the volumetric (i.e., volume-specific) rate, which is the rate of product formation per unit of bioreactor volume.
Renewable chemicals: commodity chemicals that are produced from renewable feedstocks, for example, plant materials or carbon dioxide.
Stoichiometric model: a representation of the biochemical pathways of a cell through a system of linear equations. Since the reactions are catalyzed by enzymes that are translated and transcribed from the genome, a network covering all available reactions stored in the genome of an organism is referred to as the 'genome-scaled model.'
Theoretical yield: the yield that is obtained from the complete reduction of the substrate(s) to the product. The maximum yield possible in a cell (in the absence of growth and maintenance) can be calculated through stoichiometric models of the cell, which take into account the available biochemical pathways.
Titer: the final concentration of the product in the bioreactor.
Yield: the yield of product per unit of substrate (typically glucose). The yield of

Box 3. Mathematical formulation of the TRY metrics

In general, the production rate (R, in g/h) is the product of the volume-specific productivity [q_p, in g/(h*l)] and the volume (V, in l). In turn, q_p is the product of the biomass-specific production rate [r_p, in g/(g biomass*h)] and the biomass concentration [x, (g biomass/l)]. The full equations for the production rates for the product (P), substrate (S), and biomass (x) are displayed in Equations I–III. In the case of biomass, r_x is substituted by the growth rate μ (1/h).

$$R_P = q_P \times V = r_P \times x \times V \quad [I]$$

$$R_S = q_S \times V = r_S \times x \times V \quad [II]$$

$$R_X = q_X \times V = \mu \times x \times V \quad [III]$$

The production rates can be used to calculate the product yield (Y_{sp}, in $g_{product\ produced}/g_{substrate\ consumed}$) and the biomass yield (Y_{sx}, in $g_{biomass\ produced}/g_{substrate\ consumed}$) as displayed in Equations IV and V.

$$Y_{SP} = \frac{R_P}{R_S} \quad [IV]$$

$$Y_{SX} = \frac{R_X}{R_S} \quad [V]$$

The product titer (c_P, in $g_{product}$/L) is the most reported of the three TRY metrics and is essentially the concentration of the product in the culture or the mass of the produced product (P) per volume (V, in L):

$$c_P = \frac{P}{V} \quad [VI]$$

The produced product is the product of the production rate and time (t, in h):

$$P = R_P \times t = q_P \times V \times t = r_P \times x \times V \times t \quad [VII]$$

Equation VII highlights the intrinsic problem of using the titer alone to compare production processes. To increase the product produced, and with that the titer, a longer cultivation time or higher biomass concentration is sufficient. This can often be achieved by changing the cultivation method, for example, from batch to fed-batch. More relevant for the comparison of different microbial engineering approaches is therefore the biomass-specific production rate (r_p).

the product may vary during a fermentation process, but for technoeconomic evaluation, it is generally the overall yield that is important. This is defined by the difference of initial and final product (mass) divided by the difference of initial and final substrate (mass). It is important to use the mass of the product and substrate for the yield calculation because the volume of cultivations usually changes over time (e.g., evaporation, pH adjustment, antifoam).

but often with an increasing trend. Thus, if you increase the fermentation time or ensure that there is a higher biomass concentration, the final titer will increase. The titer is therefore an integrative metric and as such does not say much about either the performance of the cell factory or the fermentation process. The titer is still important, however, as a low titer may cause challenges in the downstream processing where the product needs to be purified, and the titer will therefore impact the overall economics of the process. But if the two other TRY metrics are optimized, it is normally possible to design a fermentation process that results in

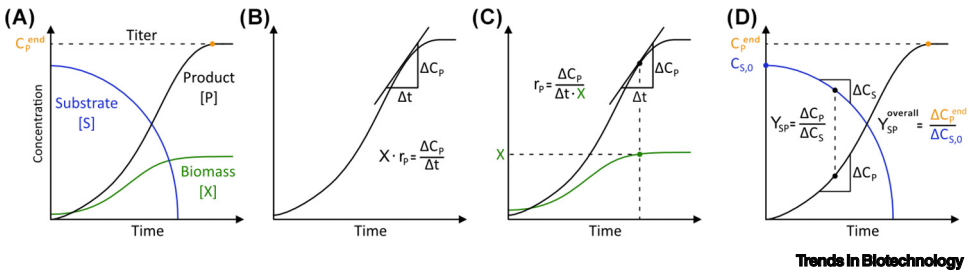

Figure 1. Titer, rate, and yield (TRY) metrics in a batch cultivation. (A) Titer (c_p^{end}) is the final product concentration in the reactor. (B) The volumetric productivity (x r_p) is given as the slope of the tangent to the product concentration at any time. (C) The biomass-specific productivity (r_p) is given as the volumetric productivity divided by the biomass concentration at the same time point. (D) The yield is given by how much product is formed per unit of substrate (typically glucose) used. There is a distinction between the yield at any time point (Y_{sp}) and the overall yield ($Y_{sp}^{overall}$) that is given as the ratio of the final titer and the initial substrate concentration ($c_{s,0}$), assuming that all substrate is consumed.

a high titer, unless the product is toxic for the cells. In the case of the product being toxic, it may be necessary to design a process having *in situ* separation or compromise on the final product titer.

(ii) Rate represents the productivity of the cell factory, and, as mentioned earlier, there is a difference between biomass-specific (product per biomass and time, r_P) and volumetric (i.e. volume-specific) productivity (product per volume and time, q_P) (Figure 1B). The biomass-specific productivity defines the performance of the cell factory, and it is the most relevant for comparing different cell factories, for example, in connection with strain development, as it directly specifies the catalytic efficiency of the cells. It is, however, quite difficult to measure the biomass-specific productivity as it will require measurement of both product and biomass concentrations throughout the fermentation process (Figure 1C). In **chemostat** cultures (Box 2), where a steady state can be obtained, it is easier to obtain information about the biomass-specific productivity, and data from chemostat cultures are therefore well suited for the design of a fermentation process (see later). For the overall evaluation of a fermentation process, the volumetric productivity is, however, more useful as it directly specifies how much product can be produced per unit of reactor volume per unit of time, and it is therefore directly linked to the required capital investments in the production plant. In technoeconomic terms, this means that the rate has an impact on the depreciation costs, which often will account for a significant part of COGS.

(iii) Yield (product produced per substrate consumed, Y_{SP}) represents the amount of product produced per amount of substrate used (Figure 1D). Normally, the major constituent in the substrate, and the major cost, is the carbon source, typically glucose (derived from starch) or sucrose. Yield defines how efficiently the carbon source is converted into the product, and if the yield is optimized, it indirectly means that the flow of carbon to by-products is minimized. Using a **stoichiometric model** (a representation of the cellular pathways by linear equations), it is possible to calculate the maximum yield possible in a cell (in the absence of growth and maintenance) that represents an upper boundary for the yield, which can be used to estimate the COGS even before the start of a project. In a fermentation process, some of the glucose must be used first for the formation of biomass, the catalyst, and then for its maintenance. It is therefore not possible to attain the maximum yield. This means that there often is a trade-off between product yield and biomass yield. In technoeconomic terms, the yield is very important as it directly defines the substrate costs, which, for commodity products such as ethanol, can account for more than 50% of the total costs. Quite often researchers report the yield of product over biomass (product/biomass) and in some cases wrongly refer to this as titer. This yield is, of course, also interesting as it directly specifies the trade-off between product and biomass, but from a technoeconomic perspective, it is less relevant.

Bio-based production of commodity chemicals

Despite many efforts, there have been only a few successful developments of novel bioprocesses to produce commodity chemicals beyond the classical production of ethanol and citric acid. Among the few processes developed are the following:

- PDO is a platform chemical that can be used to produce polymers. DuPont developed a bioprocess to produce this chemical due to a need for it in the production of the polymer Sorona, which is used for the manufacturing of fabrics, carpets, and many different plastic-based materials. The bioprocess is based on an engineered *Escherichia coli* [5], which DuPont developed together with Genencor, and the production process required a new reactor design, which was developed in collaboration with Tate & Lyle.
- 1,4-Butanediol is another platform chemical that can be used to produce polymers, for which Genomatica developed a production process. The company used an engineered *E. coli* for

their production [6]. *E. coli* is well suited to produce diols, as these are not toxic for the cell, and it is possible to obtain very high rates of production using this bacterium. Genomatica developed a large-scale production process for producing 1,4-butanediol together with Novamont and established a production plant for 30 000 tons annually. Through licensing the technology to Cargill, the production of this chemical will further expand to more than 100 000 tons annually through production at a newly developed plant.

- Isobutanol is valuable as a biofuel, either directly as a blend into gasoline or through polymerization to isobutylene that can be further converted to isoparaffinic kerosene that can be used as a jet fuel. GEVO developed a bioprocess to produce isobutanol using an engineered yeast and a proprietary process for continuous removal of isobutanol from the fermentation process [7]. The latter was important as isobutanol is very toxic for yeast (and other microorganisms). The process enabled the production of sustainable aviation fuel (SAF), but also production of a wide range of other chemicals that can be derived from isobutylene.
- In the 1990s, Cargill developed a process for the polymerization of lactic acid to polylactate (PLA) that was found to have some good polymer properties, such as low melting point, high strength, low thermal expansion, and good layer adhesion, as well as its biodegradability. Lactic acid was traditionally produced by lactic acid bacteria that can produce this acid with high rates and yields, but as these bacteria cannot tolerate low pH, the resulting product is lactate salt. The salt can be converted to the acid form, but this requires very large amounts of inorganic acid and results in the formation of large amounts of gypsum as a by-product. Cargill therefore developed a yeast-based process that could enable production at low pH whereby the acid form could be produced directly [8]. PLA is today the biopolymer being produced in the largest volumes, and expansion of the market has been supported by Corbion who are developing a novel process that enables low-cost production of the acid form of lactic acid by fermentation with traditional lactic acid bacteria.

To illustrate how the TRY metrics impact the technoeconomic performance of a fermentation process, we consider two examples: (i) the production of ethanol and (ii) the production of PDO. There are different types of processes used for ethanol production, but let us consider the so-called dry mill process that is widely used in North America. In this batch process, processed starch is added to the reactor together with enzymes and yeast cells. The enzymes ensure starch degradation to glucose used by the yeast to produce ethanol. This means that the process is started with a high glucose concentration, and over the process glucose is converted into yeast, ethanol, glycerol as a major by-product, and carbon dioxide (Figure 2A). In this process, the cells acquire ATP required for growth by the formation of ethanol, whereas they must produce glycerol to dispose of NADH formed in connection with biomass formation. Glycerol production is therefore stoichiometrically coupled with biomass production.

At the beginning of the fermentation (phase 1), there is rapid cell growth and associated production of biomass, ethanol, and glycerol, with almost complete stoichiometric coupling between the three products. Phase 2 of the fermentation is a transition phase toward phase 3, where cell growth stops, but the cells are still converting glucose to ethanol. In this phase, the ethanol concentration is so high that cell growth is inhibited, and the cell is therefore solely producing ethanol to get sufficient ATP to maintain cell integrity. As there is no biomass production, there is also no glycerol produced in this phase. As ethanol is the sole product in phase 3 of the fermentation, the ethanol yield is at (or close to) the maximum **theoretical yield** of 0.51 g ethanol/g glucose, whereas in phase 1, it is lower (typically about 0.40 g ethanol/g glucose). In phase 1, the biomass yield is about 0.10 g biomass/g glucose, whereas it is zero in phase 3. The yields are therefore varying during the fermentation process (Figure 2B). It is also clear that there is a trade-off

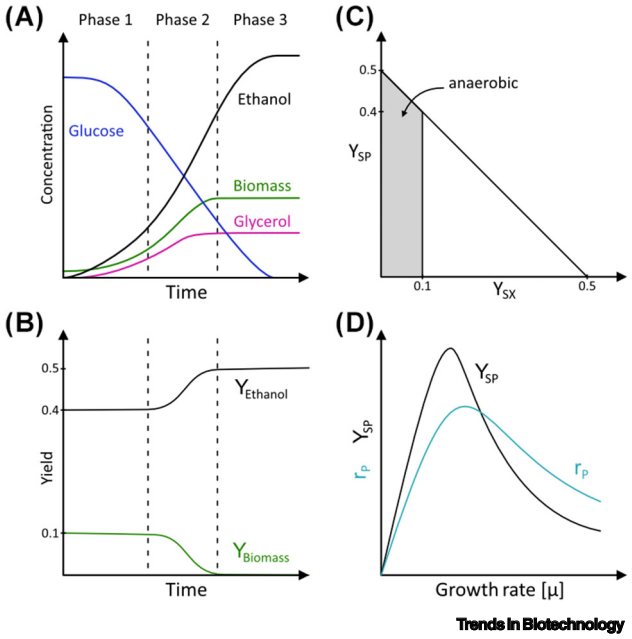

Figure 2. Trade-off between product and biomass formation. (A) Typical time profile of the ethanol production process with biomass, substrate, product, and by-product concentration. For illustrative purposes, the process is divided into three phases. (B) Time profile of biomass and product yields during an ethanol fermentation process. (C) Trade-off between ethanol yield (Y_{sp}) and biomass yield (Y_{sx}). The left part is for anaerobic ethanol production. For the sake of completeness, we added also the trade-off for aerobic growth to the right. At aerobic growth of yeast, there is normally a dramatic shift from ethanol production to no ethanol production when the specific glucose uptake rate decreases between a certain value, that is, from an ethanol yield of about 0.4 g/g to zero, and the yield range between these two values is only obtainable in a very narrow range of specific glucose uptake rates. (D) Typical profile of the biomass-specific productivity (r_p) and yield (Y_{sp}) of penicillin on glucose versus specific growth rate (μ).

between ethanol production and biomass formation (Figure 2C), which is a typical scenario in microbial fermentations. To get to a high product yield, you have to sacrifice the formation of biomass. However, since the biomass is the catalyst, a too-low biomass concentration will result in low volumetric productivity and hence poor use of the bioreactor. For ethanol production, it is fortunate that even for fast biomass growth, there is still a relatively high ethanol yield (Figure 2C), but this is due to the coupling between product formation and cellular growth in this anaerobic process.

If we consider some real numbers for the ethanol production process, the maximum specific growth rate of yeast is about 0.35 h^{-1}, and with a biomass yield of about 0.10 g biomass/g glucose, the specific glucose uptake rate is 3.5 g glucose/g DW/h. With an ethanol yield on glucose of about 0.4 g/g, this translates to a biomass-specific ethanol productivity of 1.4 g ethanol/g DW/h. Initially, the biomass concentration is low, but it may increase to about 10 g biomass/l at the end of phase 1, and the volumetric productivity at this stage is therefore about 14 g ethanol/l/h. This is a very high volumetric productivity and is hard to match for any other biotech process. In phases 2 and 3 of the fermentation, the specific glucose uptake rate will begin to decrease and along with the specific ethanol production rate. However, as there is still some increase in the biomass concentration in phase 2, the volumetric productivity may be kept at this high value. Through phase 3, the specific ethanol production rate will begin to decrease, and at the end of phase 3, the volumetric productivity will also decrease. However, the yield in this phase is high, and it is therefore important to continue the process as the yield is important for the overall economics of the process. This illustrates the dilemmas often faced when designing an optimal process. If we compare with the aerobic growth of yeast, where the specific growth rate is about 0.40 h^{-1}, and with a biomass yield of about 0.50 g biomass/g glucose, the specific glucose uptake rate here is 0.8 g glucose/g DW/h, that is, much lower than for the anaerobic process.

For production of PDO, DuPont (now Dow) reported a volumetric productivity of 3.5 g/l/h, a yield of 0.51 g/g, and a final titer of 135 g/l [5]. Even though this productivity is very high, it is still fourfold lower than the volumetric productivity that can be obtained for ethanol production. PDO is, however, also sold at a higher value than ethanol, with a price of about 1.8 USD/kg, which is higher than the typical ethanol price of about 0.9 USD/kg. The theoretical yield of PDO from glucose is 1.3 mol PDO/mol glucose, corresponding to 0.63 g PDO/g glucose, and the DuPont process is operating at about 80% of the maximum theoretical yield. It may be possible to find alternative pathways to increase the theoretical yield up to 1.5 mol PDO/mol glucose [9], but this will require optimization of this pathway to ensure the same high TRY as reported for the DuPont process.

The aforementioned TRY metrics for the production of ethanol and PDO indicate the range required for these metrics in establishing a new process for the production of a commodity chemical, and it is important to evaluate early on the maximum theoretical yield of the process and to evaluate if it is possible to obtain a higher volumetric productivity.

Bio-based production of fine chemicals

There are several successful examples of the development of novel bioprocesses to produce fine chemicals that find application as ingredients in dietary supplements, cosmetics, food, household products, and fine fragrances. Fine chemicals are the product of pathways that typically require a large energy input (ATP), which is why aerobic fermentations are often favored over anaerobic fermentations. Several companies, for example, Amyris, Firmenich, BASF, and Manus Bio, have developed fermentation-based production of sesquiterpenes that can find application as perfume ingredients in fine fragrances [10,11]. Similarly, the company Evodia recently developed a novel bioprocess to produce monoterpenes based on yeast fermentation [12]. Monoterpenes can also find application as perfume ingredients, but many monoterpenes are also valuable flavors, for example, for addition to alcohol-free beer to enhance the hoppy flavor. There are also several examples of the development of new bioprocesses to produce bioactive compounds traditionally extracted from plants, but because of supply chain issues or difficulties in obtaining pure ingredients from plant extraction, it is beneficial to have a microbial fermentation process. Examples are the yeast-based production of resveratrol [13] and opioids [14] that are either launched or are in the process of being scaled up. Many of these bioactive compounds can find applications as active pharmaceutical ingredients or directly as dietary supplements.

COGS of fine chemicals are typically in >10 USD/kg and can in some cases be significantly higher. Fine chemicals also include antibiotics, and the production of these chemicals by microbial fermentation has been a hallmark of industrial biotechnology. Some antibiotics are still sold at high prices, whereas penicillin has become a semicommodity product with a total production volume exceeding 60 000 tons and a price of about 10 USD/kg. Compared with the production of ethanol anaerobically, there is normally a trade-off between growth and product formation, such that there is no product formation at maximum growth, as is the case for the aerobic growth of yeast (Figure 2C). For these processes, it may be necessary to either engineer the cell to couple product formation with growth or restrict biomass production, for example, by using a fed-batch process where the substrate is kept at a low level in the bioreactor (Box 2 and Figure 3B). This is used to produce many antibiotics, for example, penicillin, and there is also a complex trade-off between growth and product formation for these processes. Thus, a typical profile for the biomass-specific penicillin productivity versus the specific growth rate of the biomass shows an optimum at a certain (low) specific growth rate (Figure 2D) [15]. Therefore, the penicillin yield on glucose, which is a very important factor for the economics of the process, has a very sharp optimum (Figure 2D), and it is important to operate the bioreactor in a way that cellular growth

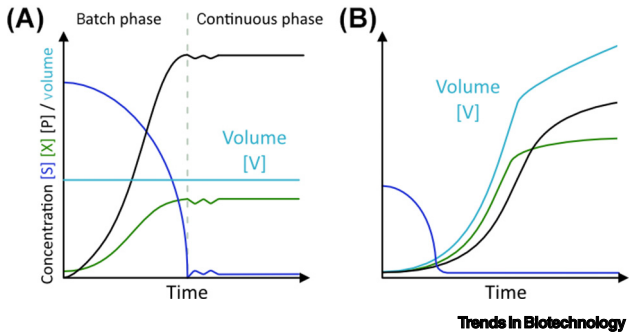

Figure 3. Concentration of substrate (S), biomass (X), and product (P) and bioreactor volume (V) over time. (A) Continuous cultivation. (B) Fed-batch cultivation.

Outstanding questions

Can the academic community agree on publishing standards that require authors to provide all three TRY metrics to enable better comparison between engineering approaches?

Should the theoretical maximal yield become a standard that is being reported as well?

Can artificial intelligence extract the missing information from previous literature, based on the provided results, and calculate missing TRY metrices?

is kept close to this optimum [15]. A similar profile has been found for the production of adipoyl-7-ADCA [16] using an engineered penicillin-producing strain of *Penicillium chrysogenum*.

Whereas continuous processes (Figure 3A) are attractive because they will not require frequent restart of the fermentation process, they are rarely applied in industry due to risk of contamination. Fed-batch processes (Figure 3B) are by far the most widely used fermentation mode in industry because they enable attaining a high final titer, and it is also possible to operate the reactor such that substrate concentration is kept at a level where rate and yield can be high.

Concluding remarks and future perspectives

Microbial cell factories can produce a wide range of products in a sustainable fashion. However, to transition from a proof-of-concept scale to an economically feasible industrial scale, certain technoeconomically relevant metrics must be considered. In a bibliome analysis, we found that academic papers mostly focus on titer, whereas yield and rate are underreported, despite their technoeconomic relevance (Figure S1 in the supplemental information online). To better compare the success and potential applications of different engineering approaches, all metrics must be considered and reported. Authors should aim to correctly report all three metrics in their papers. To avoid confusion, it would be ideal if culturing conditions were precisely described. This would allow the reader to evaluate if the calculations were made correctly and allow corrections if needed. Since the titer alone is not enough to judge the success of an engineering approach and the biomass-specific rate tends to be difficult to determine, a first improvement would be to additionally report the overall yield of a process, that is, the total product mass produced divided by the total mass of carbon source consumed.

Furthermore, the theoretical yield for a product should be considered. By comparing the achieved yields with the theoretical maximum yield, the progress of the engineering strategy can be monitored. An even better approach would be to consider the theoretical maximum yield before the start of the project and use it for a rough estimate of the process feasibility.

Journal editors and reviewers, as the safeguards of scientific literature, should remind and encourage authors to report suitable metrics in a well-documented manner. Journals with a focus on biomanufacturing can even consider including metric requirements in their author instructions.

While our recommendations can help to improve documentation of the TRY metrics and improve comparability between studies, more measures can be taken (see Outstanding questions).

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Declaration of interests

The authors declare no competing interests.

Supplemental information

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