Biochemical engineering's grand adventure

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ABSTRACT

Building on the recent revolution in molecular biology, enabling a wealth of bio-product innovations made from renewable feedstocks, the biotechnology field is in a transition phase to bring the products to the market. This requires a shift from natural sciences to engineering sciences with first conception of new, efficient large-scale bioprocess designs, followed by implementation of the most promising design in practice. Inspired by a former publication by O. Levenspiel in 1988, an outline is presented of main challenges that the field of biochemical engineering is currently facing, in a context of major global sustainability trends. The critical stage is the conceptual design phase. Issues can best be addressed and overcome by adopting an attitude where one begins with the end in mind. This applies to three principal components: 1. the bioprocess value chain, where the product specifications and downstream purification schemes should be set before defining the upstream sections, 2. the time perspective, starting in the future assuming that feedstock and product-market combinations are already in place and then going back to today, and 3. the scale of operation, where the industrial operation sets the boundaries for all lab-scale research and development, and not *vice versa*. In this way, and ideal process is defined taking constraints from anticipated manufacturing into account. For illustration, three bioprocess design examples are provided, that show how new, ideal conceptual designs can be generated. These also make clear that the engineering sciences are undergoing a revolution, where bio-based approaches replace fossil routes, and gross simplification is replaced by highly detailed computational methods. For biochemical processes, lifeline modeling frameworks are highlighted as powerful means to reconcile the competing needs for high speed and high quality in biochemical engineering, both in the design and implementation stages, thereby enabling significant growth of the bio-based economy.

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1. Introduction

It was 30 years ago today that prof. Octave Levenspiel conceived his Danckwerts memorial lecture, entitled: Chemical Engineering's Grand Adventure. There he reasoned that:

1. chemical engineering is a two-step affair, i.e. first conceiving a process scheme and then making it real
2. the first step is the most important step in the development of a process
3. chemical engineers are generally focusing on the second step
4. more attention is needed for the first step, both in academic education programs and in industrial practice.

This lecture and related article in Chemical Engineering Science (Levenspiel, 1988) has inspired a whole generation of chemical engineers to come with better process designs for novel products wanted by man. Now, with the fruits from molecular biology breakthroughs ready for harvesting, and the bio-based economy in surge, it is a good moment to reiterate his ideas, and project them onto the contemporary status and direction of the field of biochemical engineering.

The mission and essence of biochemical engineering is to deliver products that are desired by humanity, from processes where microorganisms, enzymes and/or cell lines convert renewable feedstocks, or intermediates derived from them, into added-value products in a chain of operations. Similar to chemical engineering, also biochemical engineering is a two-step activity: first conceiving a design, and then putting it into reality.

Nomenclature

Symbol			*Superscript*	
a	anabolic		max	maximum, without involvement of O_2
c	number of carbon atoms in 1 molecule C-source			
C	concentration [mol/m^3]		*Subscript*	
C*	solubility [mol/m^3]		c, CO_2	carbon dioxide
F	flow rate [kg/h]		e	ethanol
k_La	mass transfer coefficient [h^{-1}]		G	Gibbs energy
M	broth mass [kg]		h	protons
m	maintenance rate [mol/mol$_x$ h]		m	liquid
N	number of moles [mol]		N	total gas
P	pressure [bar]		n	nitrogen source
q	biomass specific reaction rate [mol/mol$_x$ h]		o, O_2	oxygen
R	reaction rate [mol/h]		s	substrate (mostly glucose)
R	gas constant [8.314 J/mol K]		p	product
T	temperature [°C]		Q	heat
V	volume [l, m^3]		w	water
y	partial pressure [mol/mol]		x	biomass
γ	degree of reduction of 1 molecule C-source			
μ	specific growth rate [h^{-1}]			

We can also acknowledge that the second part of this activity is something that has traditionally received most attention, and has resulted in global total markets for liquid biofuels, other bio-energy products (biogas, bioelectricity), food and food ingredients, feed and feed products (dairy, meat), bio-materials, and other products made in thousands of large-scale factories harnessing the power of biology.

In order to meet the requirements for the assumed growth of the bio-based markets, maintaining the products at high quality and affordable for consumers and in parallel meeting targets to limit poverty and climate change, and as well satisfy other global macro-trends, it is questionable whether the current state-of-the-art in biochemical engineering will be adequate. Instead, new practical unit operations, contacting schemes and biocatalysts need to be designed and then implemented. This creative or inventive step and the need for adequate training of the next generation of biochemical engineers will be one of the main challenges, and topic of discussion in this overview.

2. Bioprocess design

Bioprocess design is in the classical way executed from the perspective of where one starts, i.e. with the raw materials, with the experimental set-up and conditions in the lab, and with the market and technology knowledge of today. However understandable from the point of view of the engineer, this approach is inherently slow because merely based on trial-and-error and incremental steps forward, and therefore not adequate to meet ambitious goals and aggressive timelines. For better, representative design results, it is advised to revise the perspective in all these three dimensions: start with the product, the future market situation and the large scale (see Fig. 1). In brief, begin with the end in mind. This retro design philosophy will now be further elaborated in three examples.

2.1. Reversing the bioprocess value chain

A bioprocess can be depicted as a sequence of operations, run at industrial large scale, where renewable feedstock is converted and purified into a final form that meets the specifications of the client or user. Zooming in on the whole chain, it is clear that there are alternating steps of conversion to rearrange the molecular bonds, and separation to get rid of the co-products (either 'waste' or a compound that can be separately valorized) of the reaction. Usually, it is presented as a linear path from feedstock to product. However, for the design purpose it is better to start from the perspective of the final product, see Fig. 2.
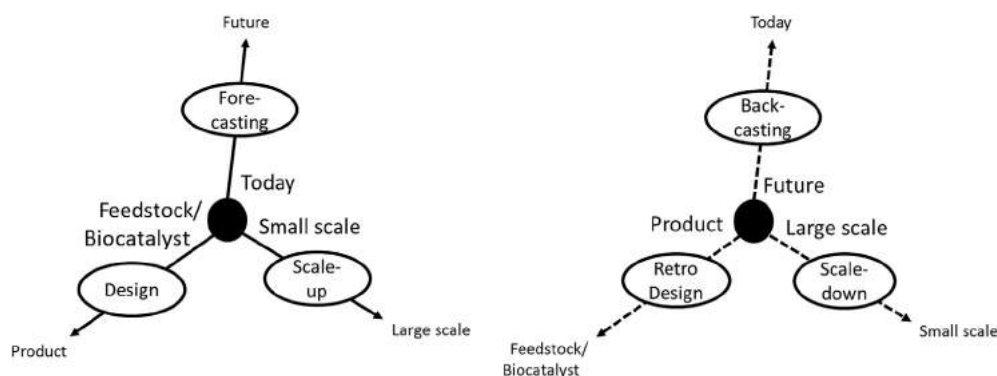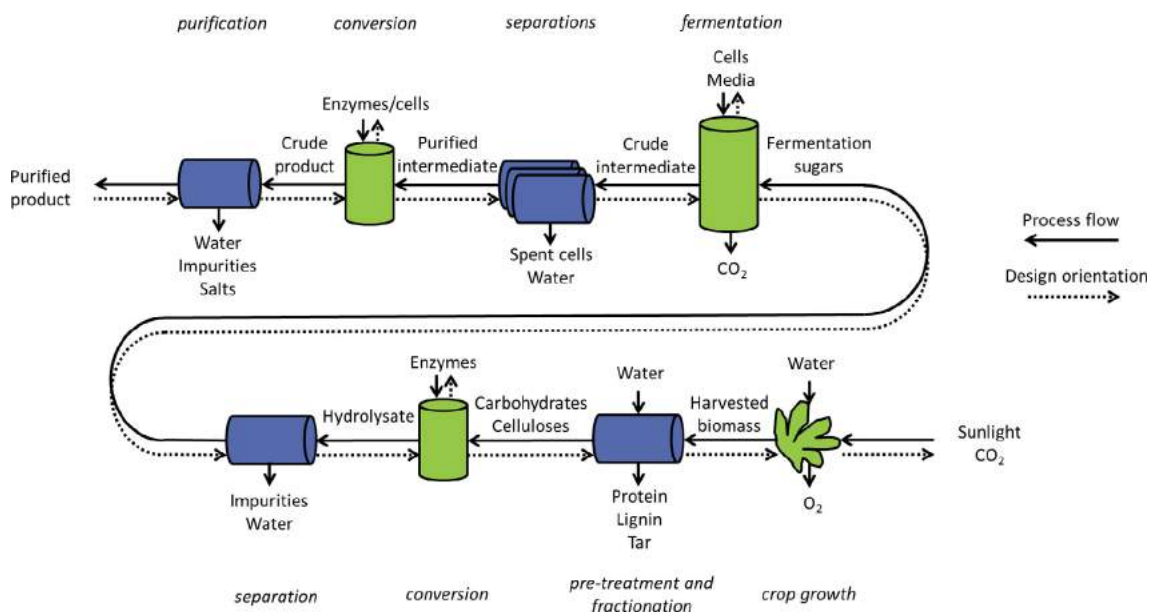

Fig. 1. Left: traditional approach in bioprocess design. Right: recommended view on bioprocess design, with a reversed perspective in three dimensions. This turns value chain design into retro design, forecasting into backcasting and scale-up into scale-down.

Fig. 2. The bioprocess value chain, with sugar hydrolysates as intermediate platform, positioned from the perspective of the final purified product. The design orientation is opposite to the process flow.

The power of this approach can be best illustrated via an example. For the production of succinic acid from sugars, a handful of companies has in recent years developed and implemented large-scale bioprocesses using microorganisms and fermentation (Cok et al., 2014). Succinic acid is a monomer for bioplastics, which has been attributed large potential to replace fossil-based plastics (Werpy and Petersen, 2004; Weastra, 2013). Most companies have selected bacteria that were already capable in nature to produce succinate at high rate and selectivity, or could be readily genetically modified to do so, at neutral pH. After the fermentation, the succinate then is converted into the free acid, which is the ingredient for the polymerization. This latter conversion requires pH shifts and results in the formation of salt as co-product, usually sulfates such as sodium sulfate or calcium sulfate dihydrate (gypsum), in amounts similar to the main product (Fig. 3). Because the global

markets for these salts have become saturated, companies have adopted solutions via additional separation/conversion operations to modify the salts and recycle the constituents to the upstream sections. This is all technically achievable, albeit at a cost. Considering the low economic margins of the monomers business, it would be preferable to directly make succinic acid at the fermentation stage, that is at pH lower than 3.5. However, in nature no suitable microorganisms were known until recently. Researchers from Reverdia (www.reverdia.com), a joint venture between the companies DSM (www.dsm.com) and Roquette (www.roquette.-com), managed to develop a yeast, *Saccharomyces cerevisiae*, using new rational genetic modification and evolution schemes, to produce the free acid with high rate and yield at pH 3 (Jansen and van Gulik, 2014). This took more development effort than for the neutral pH fermentation, but has resulted in a simpler process,
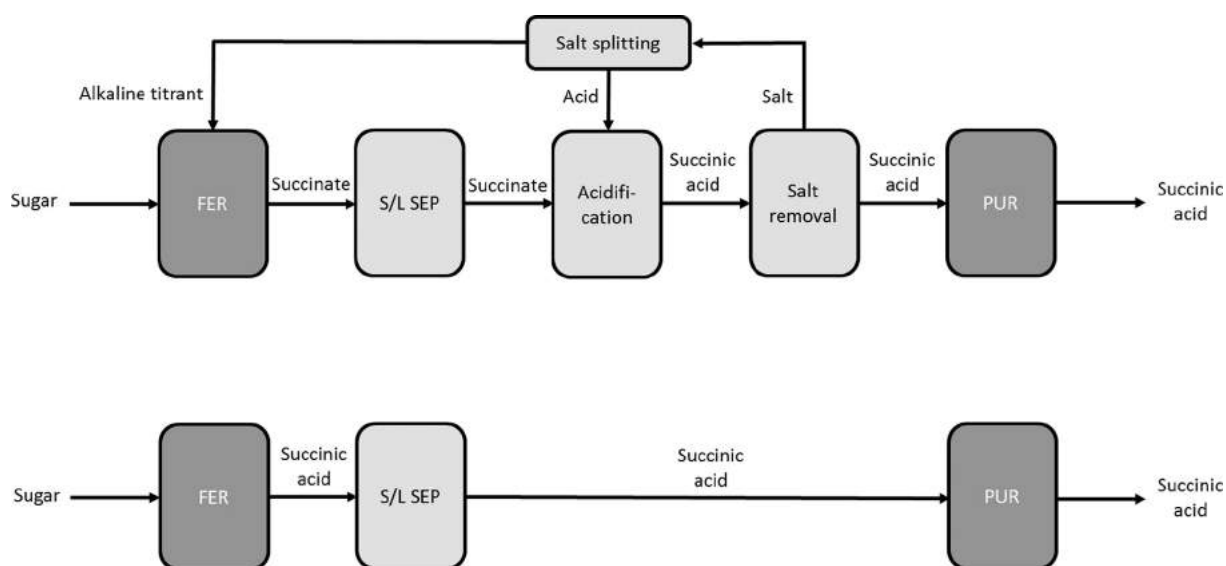
Fig. 3. Succinic acid process configurations. Top: process based on neutral pH fermentation, with a salt splitting step, e.g. via electrochemical or thermal salt treatment. Bottom: process with low pH fermentation (based on Jansen and van Gulik, 2014). FER = fermentation, S/L SEP = solid/liquid separation, PUR = purification.

and this process is now thought to be the most efficient - at least it is the least complex. Altogether, this presents a good example of beginning with the end, the desired product, in mind. The precise order of events can be summarized as follows:

– Define specifications of the product for use in bio-plastics
– Wish to prevent salts as co-product
– Simplify downstream processing, without titration steps, salt treatment (temperature cracking) and recycles
– Design the downstream operations such that the product specifications are met
– Design fermentation at pH 3, minimizing salt ion accumulation from media and titration
– Screen for various potential host strains, capable of tolerating high product concentrations at low pH and other anticipated large scale requirements, and select best host
– Design, construct and evolve the selected host (*S. cerevisiae* in this case).

2.2. Anticipating the future

A second design perspective is to start in the future, assuming that the conceived process is already serving a market. This principle can be very well illustrated by a global analysis of the bio-economy. For a proper assessment of both the opportunities and bottlenecks, some key questions should be asked first. Will there be enough renewable feedstock to allow major growth of the bio-economy, or will it be limited by global constraints? How would the most likely supply chain and market segmentation for bio-products look like?

There are many scenarios that suggest a healthy growth of the bio-based sector and this is further strengthened by the global sustainability agenda. This in the end justifies the work that is pursued within the scope of the bio-based economy. A major element is renewable energy. Here the expectations are that solar and wind in combination with water-based sources (geothermal, hydro-power) will deliver most of the future required energy, replacing part of the current fossil raw materials supply of about 12 000 million ton (MT) per year (equivalent to approximately 500 EJ). Nevertheless, clearly in many scenario's, in addition biomass will be needed, especially for liquid fuels and heat/electricity. Further, the need for carbon-based materials is expected to grow significantly and this could in part be from (lignocellulosic, marine) biomass.

It has been argued that this poses severe limitations because the planet is not able to cope with such burden and supply enough biomass to meet all the needs. However, a global analysis reveals how to fulfil the future global carbon (24 000 MT, in 2035 or 2050, depending on a fast or more moderate transition) and energy demand (estimated 500–800 EJ, for different scenario's) via biomass. The most prominent feedstocks for industrial bio-products could be based on a mix of wood, agro-residues and dedicated crops to be grown in areas that are currently not cultivated (Popp et al., 2014; Deng et al., 2015; Scarlat et al., 2015; Piotrowski et al., 2015). In Fig. 4 an impression is provided of such global mass and energy balance analysis.

Further, other investigations have shown that under the right conditions at least 50% production of energy and bio-products via biomass is well feasible without compromising nutrition needs and biodiversity, via new system optimizations (e.g. using lignocellulose) and farming/crop technologies in combination with fossil carbon capture and utilization (Piotrowski et al., 2015). In parallel, this will contribute to mitigation strategies for climate change, with the majority of the effect to be realized for heat/electricity and liquid transportation fuels, and less in plastics and other materials. For the field of biochemical engineering this grossly means a major extension of the value chain further upstream, and integral assessment for design. In fact, this represents the whole bottom part of Fig. 2. This is different from today, where the supply of sugars is merely an outlet of the food supply chain. Novel biorefinery technologies will be required, as well as innovations to convert the mix of crude bio-based feedstocks into the proper intermediates for fermentation. In order to serve global markets, the production of feedstocks should be regulated and preferably standardized to secure constant quality of the end product. Especially decentralized production with high substrate flexibility will require robust process solutions, and this whole field requires further development.

In addition to sugar hydrolysates as feedstock for fermentation, either *first generation* glucose syrups and sucrose juices or *second generation* lignocellulosic sugars, the whole picture also reveals that there are some alternative substrates. The following options are presented here:

– Syngas could provide an alternative substrate for microorganisms (Latif et al., 2014; Bertsch and Müller, 2015). Syngas can be readily produced from all kinds of biomass via thermochemical treatment, replacing the enzymatic hydrolysis step in Fig. 2. In addition, the carbon atoms and energy from lignin will stay in the main process and it does not need to be separately valorized, which is beneficial for the overall process economy.
– Bio-syngas could as drop-in to the existing fossil syngas networks also be converted into methanol, which is a potential substrate for many organisms. The advantage is further that methanol can be supplied in pure form, with limited impurities and highly concentrated (water-free).
– Methane, as natural gas or compound present in biogas, could serve as bio-based feedstock for the manufacturing of various bioproducts (Strong et al., 2016). It is a massive, low cost resource, does not contain water, and sequesters a potent greenhouse gas.
– Crude intermediates from the emerging bio-fuel platforms could be diverted to fermentation feedstocks (Weusthuis et al., 2011). Examples would be the ethanol, either in purified form or as 40% w/w from the first biorefinery distillation step, glycerol as outlet from the bio-diesel industry, or fatty acids. These compounds are more reduced than sugars and could be beneficial for the biosynthesis of reduced products.

All in all, these variations present different design options that could be beneficial in specific cases, with degrees of freedom in water content, impurity level, and degree of oxidation/reduction relative to the desired product. The benefits can be capitalized via improved key process performance indicators, as will be illustrated in the following sections.

2.3. Scale-down

The third design perspective is to start from the large-scale operation, and not from the position of the labscale worker. In the lab, given the small size of equipment usually the transport steps are not rate limiting, while at the large scale the transport paths are much longer and the overall rate of reaction is determined by transport phenomena. At the fermentation stage this is either:

- mass transfer
  - oxygen, or the syngas constituents H_2 and CO, from the gas phase to the liquid phase and from there to the microorganism, and *vice versa* removal of CO_2 away from the cells
  - release of sugar monomers from solids and polymeric substances and transfer from the solid to the liquid phase
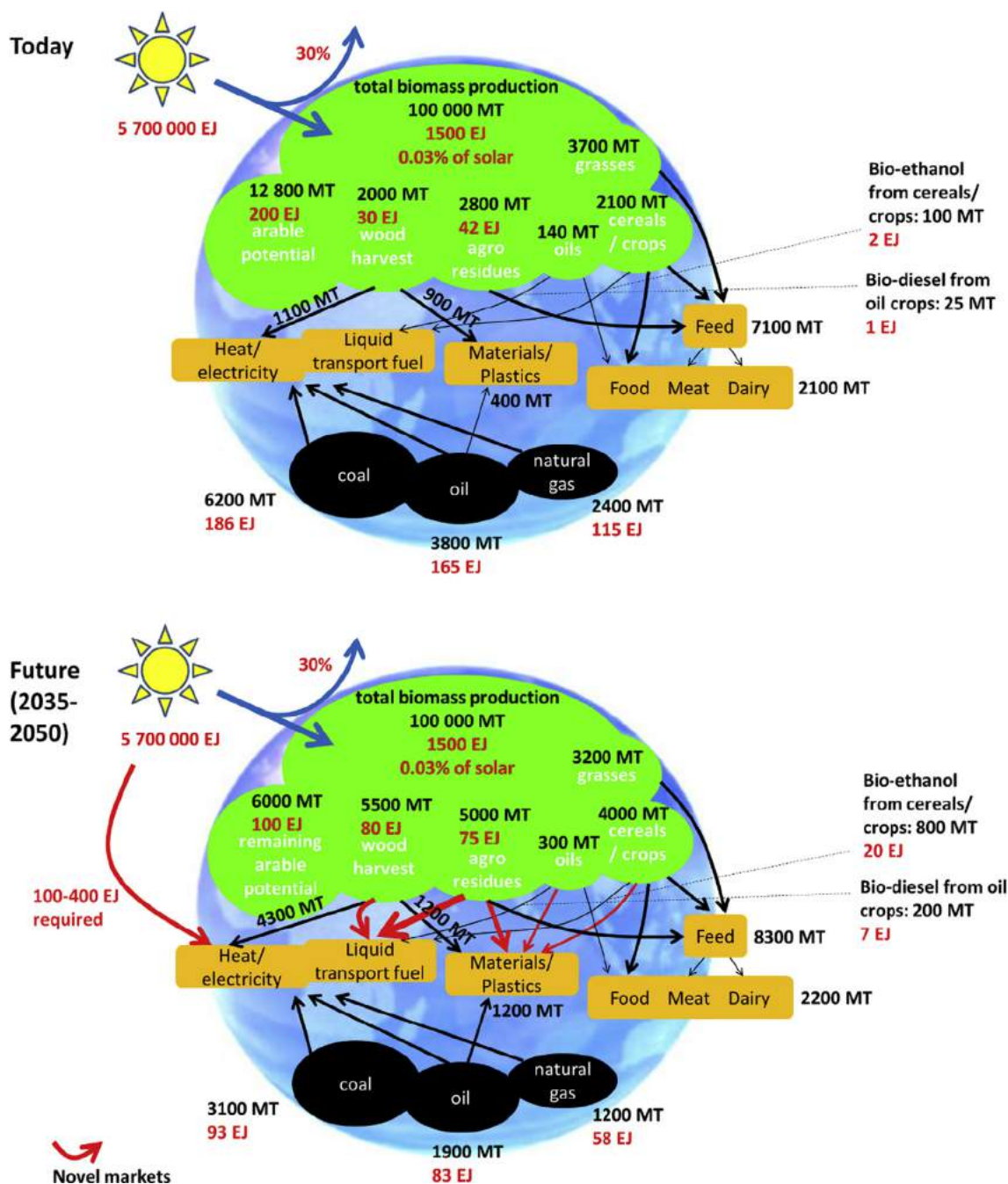
Fig. 4. Global overview of major annual renewable and fossil carbon and energy sources, and their main utilizations today (top) and projected for the future (bottom: scenarios for 2035, with fast transition, and 2050, with moderate pace of transition). Even though today only a minor fraction of the solar energy is harvested in biomass (crops, grasses, trees, marine algae) there is a large amount produced every year, of which about 10% is used for current needs, and there is potential to elevate to 25% for human exploitation. This may, in combination with renewable energy sources, in the future replace up to 50% of the fossil feedstocks for energy purposes, and also a significant percentage for materials such as plastics (Popp et al., 2014; Deng et al., 2015; Scarlat et al., 2015; Piotrowski et al., 2015; FAOSTAT, 2011; IEA, 2013; IPCC, 2013, own calculations]. It is further noted that a fast deployment of electromobility might enable a shift from liquid (bio)fuels to (bio)electricity, thereby reducing pressure on the biomass generation system.

- heat transfer (cooling)
- macromixing of the supply of limiting substrate, dosed in one place of the bioreactor
- separation of the gas bubbles from the liquid, or
- a combination thereof.

As a result, at labscale the regimes for fluid flow and reaction are likely not the same as in the large scale. Therefore, findings and performance achieved in the lab are not automatically translatable to the factory: from the screening of improved strains generated by the genetic engineering department then *champions* in the lab (superior performers) may be identified and selected, but they turn out as *losers* in the plant. The proper way to do the screening and selection is to apply downscale simulators that mimic the large-scale cellular environment in the lab. Herein, cells are exposed to rapid fluctuations in the concentrations of limiting substrates, dissolved oxygen levels, pH, temperature, shear rate and other factors that are deemed relevant. The design of the

scale-down simulators is not trivial is should be guided by a detailed analysis of the large-scale first. Even in the early stage of the development of a new process for which no plant is yet running, such analysis is possible based on the accumulated information in the open literature. It is not needed to know the exact conditions – 30% inaccuracy can be tolerated. The literature has accumulated several different approaches, which all have their merits and are often limited by lack of detailed knowledge from the large-scale, so that gross assumptions and simplifications are applied (Ying Lin and Neubauer, 2000; Delvigne et al., 2006; Neubauer and Junne, 2010; Takors, 2012; Lemoine et al., 2015). The availability of powerful computational tools (CFD-based) in recent years has enabled high-resolution descriptions of the (anticipated) large scale processes, which can be used to design the scale-down simulator set-up and operation conditions with much higher precision (Haringa et al., 2016, 2017; Lapin et al., 2004, 2006). This will be explained in more detail later on. It is noted here that general use of this methodology still has limitations as there are only few large-scale data sets available to verify computer predictions. Given the lack of standardization in bioreactors and bioprocesses, there is a need to make more large-scale data available which requires better access to industrial facilities.

The three key elements of conceptual design illustrate that before starting to work on a particular process concept, one should define a set of criteria for the desired end point. This requires *thinking research* even before going into the lab or small pilot plant. And this also requires a proper mind-set, more remote from the daily hassle and practical boundaries, and closer to the ideal, or dream.

3. The ideal full-scale process

At industrial scale, the performance of a process is mainly determined by four indicators: yield (conversion efficiency), productivity (rate), product titer and downstream processing efficiency. Combined with cost and operational needs, these result in six key requirements for an ideal process (see Fig. 5):

1. The maximal efficiency of conversion or yield is governed by thermodynamics, where the Gibbs free energy available in the substrate should be as much as possible be harvested and preserved in the product(s). The maximal theoretical yield, that is the efficiency of conversion in the absence of oxygen, is the first indicator and the final process should stay as closely as possible to this limit. Competing routes, such as formation of biomass, generation of energy for maintenance purposes and by-product formation should be minimized where possible. Such will also have clear benefits for downstream processing.

2. The productivity is determined by a combination of the process rate, which is ruled by a transport process and not the kinetics, and the net production time, that is the real production time minus the sum of the turn-around time between batches and the lag time, or start-up time before the product is being formed. The limiting transport rate is determined by hardware, i.e. energy input via a compressor and/or agitator, area for transfer, and process characteristics such as viscosity, pressure and temperature, and the ideal is mainly set by cost constraints. Further, a continuous process would be the ideal as then the on-stream time is maximal and the volume minimal, allowing simpler bioreactor concepts; however in reality there are limitations because the microorganism has a limited stability.

3. A high product titer is a key outcome of high yield and rate on one side, and tight water management on the other side. Supplying as little as possible water via the feedstock, and possibly removing a surplus via the off-gas via evaporation will provide the ideal conditions for a high product titer. This will further facilitate the downstream processing sections, in particular the initial biomass separation and subsequent concentration steps.

4. The bioreactor should be as simple as possible, easily scalable, and have minimal maintenance requirements. This will point towards the bubble column as the most convenient workhorse, and not the stirred tank, loop reactor or fluid or packed bed concepts. However, for demanding, high-intensity processes operated in a bubble column, it could be that additional measures are required to control foaming or deliver locally extra energy input, via e.g. liquid jets or an agitator.

5. The microorganism should have ideal properties for the process, that is a. non-filamentous to avoid rheology issues that compromise transport rates, b. able to operate at high temperature to maximize cooling efficiency and kinetic reaction rates, c. robustness against high concentrations of products (incl. CO_2) and inhibitors, as well as stresses as a result of a rapidly changing cell environment, and d. capable of utilizing all parts of the supplied feedstock.

6. Integration of upstream processing, fermentation and downstream processing operations will result in a more simple overall process, even if the individual steps are sub-optimal. One particular example is the retention of biomass in the bioreactor,
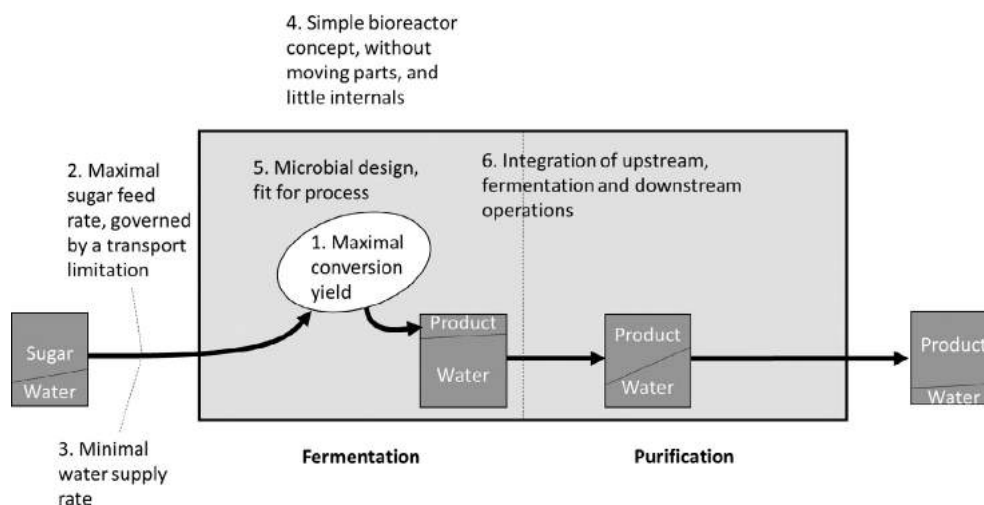

Fig. 5. Illustration of the six main process requirements for an ideal process.

which is both an advantage in downstream processing as well as an absolute need when cell growth is compromised by a small cell energy budget, strong product inhibition or highly effective metabolism towards product formation, leaving little carbon for biomass formation. *In situ* product recovery is another example, in case the product is unstable or when product toxicity for the microorganisms cannot be resolved, or when the product can leave the bioreactor in gaseous form.

4. Design examples: bioethanol, 1,4-butanediol, bakers' yeast

Let's now apply this type of thinking to three industrial fermentation cases: a biofuel, a monomer for biopolymers and a food ingredient, and try to achieve the ideal. The cases have been selected from the literature as typical representatives of the fuel, materials and food ingredients markets that they serve. The examples will make clear that via a careful design analysis there are substantial gains possible in the key performance indicators: yield (at least 5% for developed processes like bioethanol and bakers' yeast) and productivity/titer (a factor 2 or more for 1,4-butanediol). We are confident that such improvements can also be achieved for other bio-products, although every case will require a specific study to estimate the improvement potential.

4.1. Bioethanol

The biggest contributor to the bio-based economy in terms of fermentation volume is bioethanol. Nowadays (Biofuels Digest, 2013), there are about 1000 bio-refineries worldwide processing glucose syrups or cane juices, so-called first generation feedstocks, into ethanol. A novel development is the use of second-generation - lignocellulosic - feedstocks, like sugar cane bagasse and corn stover, that are now not used to extract value and potentially could overcome the perceived conflict of food versus fuel. A handful of companies have now reported to be engaged in starting up novel industrial plants to produce ethanol. The production process is more elaborate than the regular fermentation with clean sugars, because the lignocellulosic biomass needs to be thermally and/or chemically treated, and then hydrolyzed and detoxified before the sugar hydrolysates can be supplied to the fermentation stage. Part of the feedstock, notably the lignin fraction, is set aside for separate use e.g. incineration and generation of heat or bioelectricity, and another part of the material is proven recalcitrant and cannot be readily converted. This reduces the overall efficiency of the novel process. For this reason, other companies have considered revival of the gasification routes, in the past in use for production of syngas from coal to replace petroleum as the feedstock for chemicals. With this technology, in principle all of the carbon present in the lignocellulosic biomass can be converted into syngas compounds, giving a yield advantage of at least 10% compared to the sugar platform, and then via a fermentation step using acetogenic bacteria into ethanol. Levenspiel provides six criteria for an ideal syngas process from coal and this can be modified for biomass as follows:

- Only use air, water, biomass
- No N_2 leaves with the product gas
- No tar or liquid formed
- No O_2 plant to be used
- All flow streams leave at room temperature
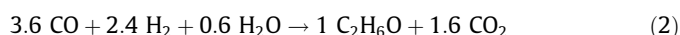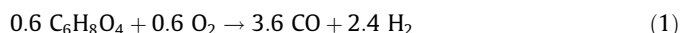- Process must be simple, practical, and easy to operate and control

For this to be achieved it is key to separate two sub-reactions: the endothermic formation of syngas from biomass, using steam, and combustion of a minor portion of the biomass with air into CO_2 and water, using the heat for the preferred reaction and heating up the fresh biomass. Levenspiel proposed a single reactor bed in which the gasification and regeneration (the RE-GAS concept) take place, operated in a periodic manner. The concept satisfies all design criteria and has been labelled superior to other set-ups.
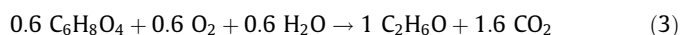
It is noted that periodic operation poses some challenges to the process and the stability of the output, as evaluated later on (Glöckner et al., 2004). Also, with pinch technology the heat recovery efficiency can be higher than 90% in multiple reactor-set-ups (Piccolo and Bezzo, 2009), but these are the topics that are then further addressed in the process development and implementation stages, i.e. outside the scope of this paper.

Regarding the fermentation stage, it is known that acetogenic bacteria are quite tolerant against varying syngas compositions and toxic compounds carried in the crude syngas, providing additional advantages. The syngas-to-ethanol fermentation has in recent years attracted strong attention and funds and led to announcements of first large-scale plants (Latif et al., 2014), based on cheap sources of syngas.

One way is to produce the syngas from renewable biomass, e.g. wood residue. The gasification of wood, $C_6H_8O_4$, via mild oxidation and the subsequent fermentation reaction, obeys the following stoichiometries:

$$0.6 \ C_6H_8O_4 + 0.6 \ O_2 \rightarrow 3.6 \ CO + 2.4 \ H_2 \tag{1}$$

$$3.6 \ CO + 2.4 \ H_2 + 0.6 \ H_2O \rightarrow 1 \ C_2H_6O + 1.6 \ CO_2 \tag{2}$$

resulting in an overall process reaction of

$$0.6 \ C_6H_8O_4 + 0.6 \ O_2 + 0.6 \ H_2O \rightarrow 1 \ C_2H_6O + 1.6 \ CO_2 \tag{3}$$

If we further assume that there is no significant formation of biomass nor by-products, and a negligible need for feedstock catabolism to generate enough energy, then the maximally achievable, theoretical, yield of product on feedstock is 0.53 g/g.

For comparison, the traditional ethanol fermentation stoichiometry from glucose reads as

$$0.5 \ C_6H_{12}O_6 \rightarrow 1 \ C_2H_6O + 1 \ CO_2 \tag{4}$$
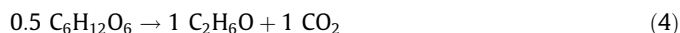
with a theoretical yield of 0.51 g/g.

There can be identified further process improvements, of which we name four:

- off-gas recycle will increase the limiting mass transfer rates of CO and H_2 and maximize the product yield
- higher temperatures in fermentation, e.g. 70 °C or higher, will ease the downstream processing for volatile compounds such as ethanol, so that distillation costs can be reduced
- the syngas composition can be manipulated by an intermediate water gas shift reaction step,

$$CO + H_2O \leftrightarrow H_2 + CO_2 \tag{5}$$

to produce either only H_2 (which is twice as soluble as CO at 70 °C, and has a 50% higher film diffusion rate, enabling faster mass transfer) or only CO (from which per mole ethanol twice as much Gibbs free energy is liberated compared to H_2)

$$2 \ CO_2 + 6 \ H_2 \rightarrow 1 \ C_2H_6O + 3 \ H_2O \tag{6}$$

$$6 \ CO + 3 \ H_2O \rightarrow 1 \ C_2H_6O + 4 \ CO_2 \tag{7}$$
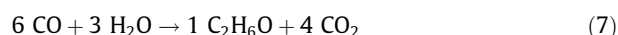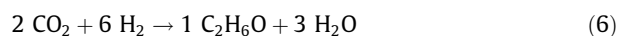
- the bacteria could be engineered to produce other products than ethanol (Latif et al., 2014; Bertsch and Müller, 2015).

The impact of a combination of the first three of these points has been assessed in a design exercise, see Fig. 6. Assuming that mass transfer is limiting the overall reaction rate, then the product of the mass transfer coefficient, k_La, and the solubility, C^*,
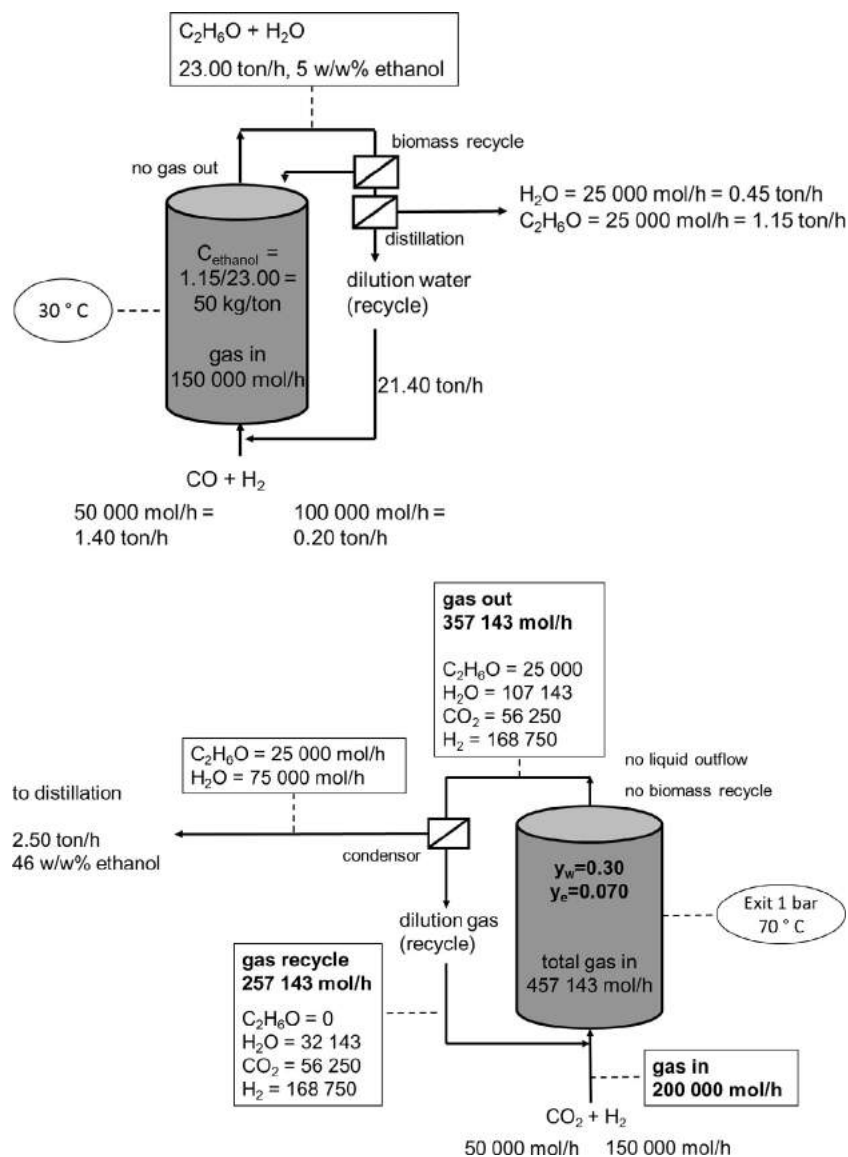
Fig. 6. Re-design to evaluate the impact of three process improvement ideas on the conversion of syngas into ethanol. Both cases produce 25 000 mol ethanol/h, where the ethanol concentration in the liquid is set at 50 g/l, which represents a toxicity limit for acetogens. Top: reference case with low gas flow rate, a mixture of CO and H_2 as gas inlet, and operated at 30 °C. Bottom: improved case with off-gas recycle and higher fermentation gas flow rate, a CO free inlet (only H_2 and CO_2), at 70 °C. Under these conditions the gas phase mole fraction of water, y_w, is 0.30, and of ethanol, y_e, 0.070.

represents the maximal productivity of the process. In the improved process, the average gas flow rate is fivefold higher, resulting in a threefold higher k_La at 30 °C. In addition, there is a temperature advantage of 2% per degree, also a factor three in total, and therefore the overall k_La is about a factor nine increased. The gain is somewhat reduced because the solubility of H_2 is lower at higher temperature, and the gas phase is more diluted in this case (C^* is 0.50 mol/m^3 at the average partial pressure of 0.67 bar H_2 at 30 °C; 0.41 mol/m^3 at average 0.59 bar H_2 and 70 °C). The resulting gain in mass transfer rate is about a factor seven, which brings main advantages as the scale of operation can be 1000's of m^3. This allows a sevenfold smaller reactor design, next to additional advantages in downstream processing regarding biomass recycle and distillation.

Altogether, the ideal hybrid thermochemical-biochemical route could present a superior route from biomass to ethanol and potentially be disruptive for the larger community currently working on the entirely biochemical process.

4.2. 1,4-Butanediol

The commodity chemical 1,4-butanediol (BDO) is nowadays produced from petrochemical sources at a volume of around 1000 kT per annum. In recent years, building on breakthroughs in metabolic engineering, bacteria have been reprogrammed to produce BDO efficiently, and the production is currently entering the commercial stage (Burgard et al., 2016) based on a fed-batch dextrose fermentation and purification via multi-stage distillation. Fermentative BDO can be further processed into biopolymers of which some types are biodegradable (e.g. PBS, poly butylene succinate). It can also be catalytically dehydrated to 1,3-butadiene (BD), a compound that is important in the manufacture of e.g. synthetic rubber but suffers from tight supply related to a growing share of shale gas to the refineries, and a higher demand of ethylene and propylene from the crackers.

There is little known about the process performance, but based on reports for 1,3-propanediol (PDO) (Nakamura and Whited,
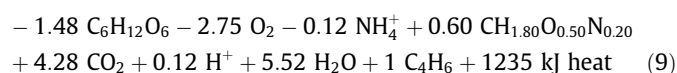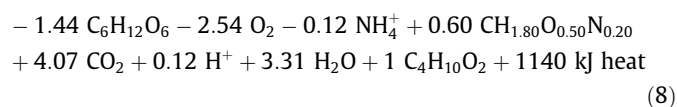
2003) we could assume that the fermentation produces BDO with a rate of 135 g/l in 67 h overall fermentation cycle time, i.e. 2.0 g/l h, at a yield 0.76 mol/mol h glucose (0.38 g/g) which is 70% of the theoretical maximum (12/11 mol/mol, 0.54 g/g). Let us now rethink the process design, and try to define the ideal process, both for BDO and for the direct fermentation of BD which will be interesting on its own but also because BD is highly volatile and could bring advantages in downstream processing, circumventing multiple distillation steps.

We assume that the fermentation of BDO will produce 50 kt per year and BD 30 kt per year, in a continuous operation, at a specific cell growth rate of 0.012 h^{-1} and a cell specific productivity of 0.020 mol product/Cmol biomass h. The temperature is 35 °C, pH 7.0 and the fermentor is operated as an air-sparged bubble column, with headspace pressure of 1 bar. Glucose solution is added as a syrup containing 720 g/kg glucose, and also ammonium sulfate as N-source to control the ammonia broth concentration. As titrant 8 mol/kg KOH is used. Further, we assume some constraints in the operation, such that the limiting glucose concentration is 0.05 mmol/kg, the dissolved oxygen concentration is 0.03 mmol/

kg, and the maximum allowable CO_2 pressure 0.05 bar. These are all numbers that may in reality vary around these assumed values, however, they are all in the ballpark of industrial reality.

From a thermodynamic analysis, including cell growth and maintenance energy requirements (see Appendix A for details) the process reactions can be evaluated as follows:

$$- 1.44\ C_6H_{12}O_6 - 2.54\ O_2 - 0.12\ NH_4^+ + 0.60\ CH_{1.80}O_{0.50}N_{0.20}$$
$$+ 4.07\ CO_2 + 0.12\ H^+ + 3.31\ H_2O + 1\ C_4H_{10}O_2 + 1140\ kJ\ heat \tag{8}$$

$$- 1.48\ C_6H_{12}O_6 - 2.75\ O_2 - 0.12\ NH_4^+ + 0.60\ CH_{1.80}O_{0.50}N_{0.20}$$
$$+ 4.28\ CO_2 + 0.12\ H^+ + 5.52\ H_2O + 1\ C_4H_6 + 1235\ kJ\ heat \tag{9}$$

From the mass and compound balances in the gas and liquid phases we can reconstruct all the rates and concentration for this process (see Fig. 7 for final results and Appendix B for calculation approach and details). As a result of this definition of an ideal process, constrained by CO_2 inhibition and minimum O_2 require-
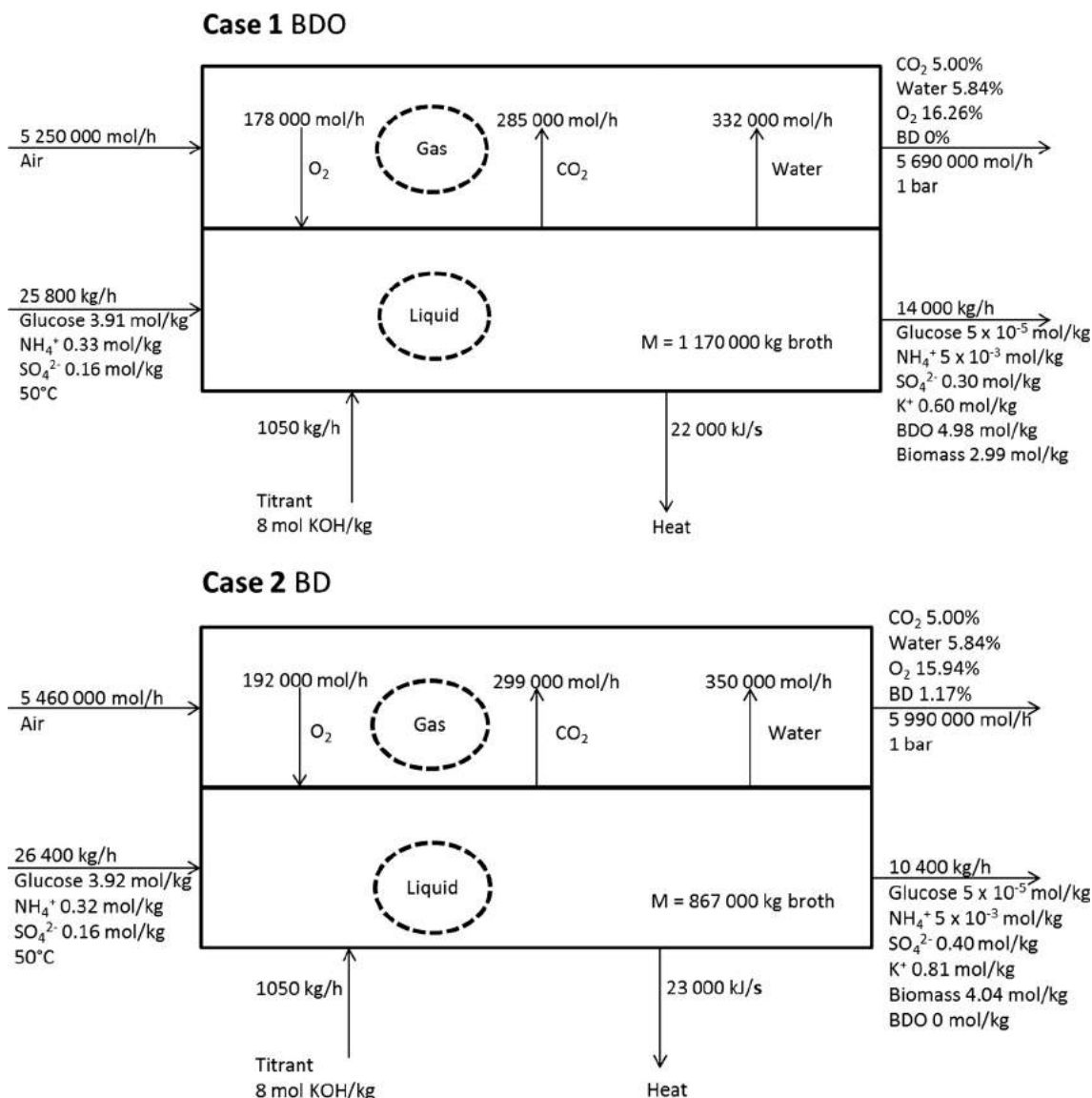

Fig. 7. In and outflows, gas and liquid composition, and reactor total broth mass, calculated in steady state for the two presented cases BDO and BD. Note that the numbers are rounded off compared to the detailed calculations presented in Appendix B.

Table 1
Key performance indicators for the 'ideal' BDO and BD processes, and comparison with current estimated values of the reported process (reference).

Case	Titer/gas fraction	Rate (g P/kg broth h)	Yield (g P/g S)
BDO reference	135 (g P/kg broth)	2.0	0.38
BDO ideal design	448 (g P/kg broth)	5.4	0.35
BD ideal design	1.17 (mol% in off-gas)	7.3	0.34

ments, the three key performance indicators yield, titer and rate are as displayed in table 1.

It is concluded that the yields will not be higher than around 70% of the theoretical maximum, however the titer and rate are dramatically increased compared to the current process. This titer for BDO may be considered as exceptionally high, and osmotic or other stress factors may provide restrictions, however, these could be good areas for debottlenecking. Further, the same framework can be straightforwardly applied with other assumptions and based on other constraints as well. Of course, this only presents an initial picture. It remains to be verified whether these reaction rates can be realized by a reasonable input of energy input required for gas compression and sparging, cooling, pumping of the fluids, etc. and also other design elements should be included such as tolerance of the microorganism against high product concentrations, downstream processing technology and operations, and the economy needs to be evaluated. And as a final verification step, it should be sorted out whether there will be significant non-ideal conditions in the large bioreactors, i.e. concentration gradients of glucose, dissolved oxygen, CO_2, pH, temperature, etc., that may compromise the performance in the large bioreactor. This can all be done to a reasonable extent via back-of-the envelope estimations in the initial stages, or simplified models for hydrodynamics (Oosterhuis and Kossen, 1984; Abel et al., 1994; Vrabel et al., 1999) but more and more such work is expected to be complemented by high-resolution computations (Montante et al., 2005; Gunyol et al., 2009; Pigou and Morchain, 2015; Delafosse et al., 2014; Wang et al., 2015), even before the engineering packages will be prepared and the activities move on to the implementation stage. This computation trend will be highlighted in the next example.

4.3. Bakers' yeast

Bakers' yeast is one of the oldest industrially made bio-products and still serves the global bakery industry with active yeast. The yeast, *S. cerevisiae*, is produced from molasses in bioreactors of a few 100 m³ volume, in fed-batch operation to minimize unwanted formation of ethanol as co-product. It has been reported (George et al., 1998) that the biomass yields can be 5–10% reduced upon scale-up, related to futile cycling of ethanol and other overflow metabolites, as a result of local high sugar concentrations (Crabtree effect) and/or low oxygen concentrations (Pasteur effect) in the large bioreactor. Scale-down of the bakers' yeast fermentation has been under study since many decades (Sweere et al., 1988a, 1988b), but in the authors' view until today it has never been executed based on a quantitative analysis of the large scale and therefore it can be questioned how representative the results from these scale-down efforts are. Based on a regime analysis usually two metabolic regimes have been proposed, one with high substrate consumption rate, mimicking the zone close to the molasses feeding point, and one more remote with nutrient limitation or starvation. A similar analysis can be made on the availability of oxygen, and one of the other possible critical scale-dependent factors in the environment of the cells. Some authors have suggested to apply two interconnected stirred tanks of which only one is fed or aer-

ated (Sweere et al., 1988a), other propose a stirred tank equipped with an external circulation loop in which the conditions can be varied (George et al., 1993, 1998), and yet others propose on-off feeding or aeration control in a single stirred tank (Pham et al., 1998; Suarez-Mendez et al., 2014). The volume ratio of the units, and the broth recycle and feed oscillation rates have usually been proposed based on intuition, or mere an educated guess. A more detailed analysis on the same process executed in a 30 m³ scale bioreactor (Larsson et al., 1996), has revealed that the cells are moving in at least three metabolic regimes: one with glucose consumption and ethanol overflow, one with only glucose respiration, and one with both glucose consumption and ethanol re-assimilation. Only recently (Haringa et al., 2017), applying an Euler-Lagrange computational frame, it has been revealed that a three-compartment set-up of ideal stirred tank reactors, of which only one is fed with glucose and with calculated volume ratio and high recycle flow rates, mimics the metabolic rates and their dispersion much better than any other so far proposed. Fig. 8 shows details of this design and operation information, which is different from previously proposed scale-down set-ups in the literature.

The key scale-down objective is to maintain similarity, i.e. keep at both the large and small scales the physical and physiological conditions similar. In reality there are a couple of phenomena that occur in parallel, such as gradients of the glucose and oxygen concentrations and the shear rate. Experimental studies should clarify both the individual cell responses and the response of the whole cell population. Especially it has been concluded that the time-scale of fluctuations in the environment of the microorganisms should reflect the mean liquid circulation time, and not the usually applied 95% mixing time, that is 4–5 times longer and only presenting worst-case conditions. This will pose a challenge to the experimental community, to design a new generation of scale-down simulators that allow physiological oscillations with a time-scale of 10–20 s, and not minutes. One noteworthy opportunity is to apply micro-fluidic methods, that in principle allow tight control of the environment of cells (Uhlendorf et al., 2012; Grünberger et al., 2014). In such equipment, rapid fluctuations as observed in large bioreactors should be experimentally feasible.

5. Reconciling speed and precision: lifeline modeling

The need to bring bioprocess innovations faster to the market is in apparent conflict with the need for better process designs and higher precision in implementation: on one hand, a higher quality of engineering design packages usually requires more time, and on the other hand a higher speed compromises the quality. It has been long recognized that mathematical models can bridge these conflicting requirements, because a good model will bring focus in the experimental part of research, with less empiricism, and more rational, directed steps forward. Of course, upfront a good model requires an investment of time and effort, and the more comprehensive the models are, the more it will cost. Nevertheless, this initial investment will enable a shorter path to the end result, with less trial-and-error and rework. Modeling currently takes significant time because all models that are developed are unique 'R&D models'. Once advanced software packages will become available that automate a large part of the current modeling work, the effort to build and run such models is expected to become much smaller. Without giving an overview on the merits and limitations of the various modeling approaches (e.g. reviewed by Kerkhoven et al., 2014 for kinetics and Gunyol and Mudde, 2009, and McClure et al., 2016, for hydrodynamics), it can be concluded that brute-force computational approaches are gradually taking over the classical paths based on simplification schemes.
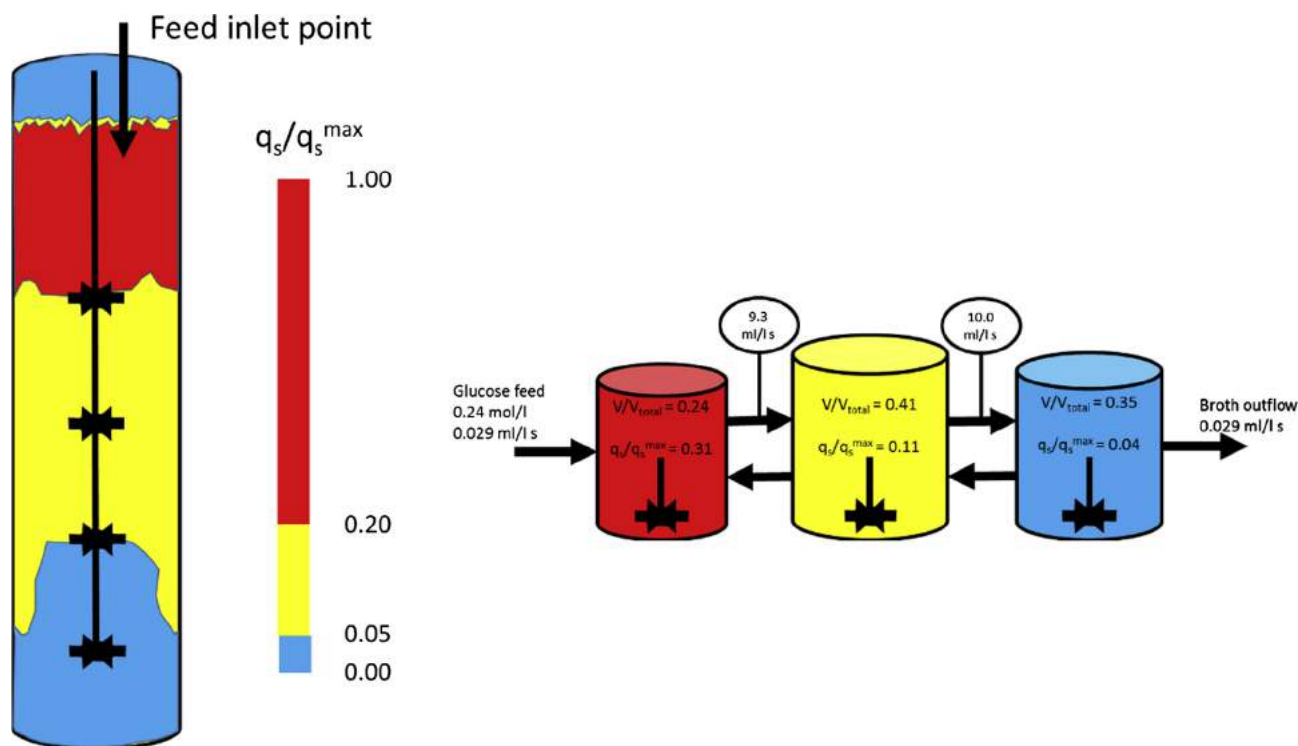
Fig. 8. Large-scale analysis and CFD-driven scale-down simulator design for *S. cerevisiae* fermentation. Left: 30 m^3 bioreactor, equipped with four Rushton turbine impellers and operated with air supply from below the bottom impeller and a glucose feed inlet point just beneath the liquid surface (Larsson et al., 1996). There are three metabolic regimes: 1. glucose consumption with ethanol overflow metabolism (when $q_s/q_s^{max} > 0.20$, denoted in red), 2. glucose consumption ($0.05 < q_s/q_s^{max} < 0.20$, yellow), and 3. glucose consumption together with ethanol re-consumption ($q_s/q_s^{max} < 0.05$, blue). Right: Recommended scale-down design applying an Euler-Lagrange CFD model (Haringa et al., 2017). There are three compartments, representing the key metabolic regimes concluded from the large-scale process, with feeding in only one compartment, and recycle of fluid between the vessels. Each reactor is operated as an aerated, ideal stirred tank. It is noted that the scale-down simulator operates here in continuous mode, while the real process is fed-batch. The advantage of continuous operation in the lab is that the conditions are better controlled, and in steady state there is more time for comparative experiments, testing e.g. sensitivities around the working point. However, it is also possible to operate the simulator in fed-batch mode, but this leaves shorter time for extensive testing. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

A major simplification has been the assumption that all cells in a population perform equally, although it has been known for a long time that there are stochastic variations in the phenotype, made visible for example via analysis of gene expression and morphology of individual cells. In recent years, with fast progress in single-cell analytical and modeling techniques (Lencastre Fernandes et al., 2011), it has also become clear that in bioprocesses there are cellular sub-populations with different metabolic reactions and fluctuations in their rates (Lidstrom and Konopka, 2010; Müller et al., 2010; Delvigne et al., 2014; van Heerden et al., 2014). Such variations have a major impact on productivity and yield of bioprocesses. New metabolic engineering strategies, in combination with tuning of the cellular environment, are required to control the heterogeneity towards improved population and bioprocess performance.

Lifeline, or Euler-Lagrange, modeling (Lapin et al., 2004, 2006; Haringa et al., 2016), in combination with experimental validation, have already demonstrated value, as e.g. shown above in the scale-down of the bakers' yeast fermentation. One of the main current limitations is the availability of suitable experimental data from industrial large scale, needed for proper validation of the model outcome. The 30 m^3 bioreactor in Stavanger, Norway, has been extensively used in many fermentation studies since the early 1990s (Larsson et al., 1996; Vrabel et al., 1999, 2000, 2001; Enfors et al., 2001) and could be considered as the exception to the rule. The rich data set from these experiments has not been exhaustively used yet and could further serve the computational advancement. One example is the loading-flooding transition of the impellers, and flow instabilities, that have been described in experiments (Noorman, 2011; Lee and Dudukovic, 2014) and form

a good challenge for computations. Another is the tuning of the glucose uptake kinetics to capture all the observations from short and longer time-scales and feed inlet positions. Having said this, it is also clear that the current state-of-the-art still shows limitations on appropriately capturing bubble phenomena, i.e. break-up and coalescence (Witz et al., 2016), and rheological impacts (Arratia et al., 2006) caused by the filamentous form of certain microorganisms, extreme cell densities, the formation of polymeric products (e.g. xanthan gum) and/or the supply of cellulosic feed-stocks (Hou et al., 2016).

However, it also can be said that despite these shortcomings, the knowledge and implementation of the kinetics (computational *reaction* dynamics) is lagging behind to the understanding of the multiphase fluid flow. More important than solving hydrodynamic issues would be to get clearer insight in metabolic dynamics under large-scale operation conditions. Especially population heterogeneity (Lidstrom and Konopka, 2010; Delvigne et al., 2014; Martins and Locke, 2015; Heins et al., 2015; Delafosse et al., 2015), a natural feature of cultures but aggravated by a changing cell environment and other stress factors, requires urgent attention as it presents a major determining factor for the overall performance. Overall, an appropriate balance needs to be established with capturing key metabolic features on one hand, and sufficient simplicity of the governing model equations on the other hand. For this purpose, lifeline models form an excellent framework for development (Lapin et al., 2004) and this would require more attention. It has been suggested to apply metabolically structured models to describe a handful of essential pools inside the cells, related to the main catabolic pathway, storage compounds, rate-limiting enzymes in the product pathway, the energy (ATP or

energy charge) and redox (NADH/NAD ratio) levels in the cell. Recent research on *S. cerevisiae* and *P. chrysogenum*, where the cells were exposed to rapid feast-famine cycles in labscale bioreactors (Suarez-Mendez et al., 2014; de Jonge et al., 2011) has revealed that especially the storage compounds play an essential role in the metabolic stability, and robustness of the bioprocess in large-scale bioreactors. Under feast conditions storage pools are rapidly filled, whereas the glycolytic intermediates are re-mobilized in the famine phase to keep the metabolic network, and the product pathways, sufficiently fueled (see Fig. 9).

Lifeline models can now be applied to large-scale bioreactor computer models where 100 000's of volume elements and millions of individual cells can be computed in parallel, in a dynamic fashion that captures all variations related to chaotic flow patterns and population dynamics. Metabolic structuring, via e.g. lumped pools, may add another factor 10 in model size. This all may be computationally challenging today, but it is expected that the computational capabilities will further evolve to enable such model expansions in the near future. Proper visualization will be important to display all relevant features of such simulation. As an example, in Fig. 10 snapshots are provided of a large bioreactor (150 m^3 volume), equipped with a turbine impeller and two axial, downward pumping impellers, in which the lifelines of 1000s of individual cells are monitored in a fine computational mesh.
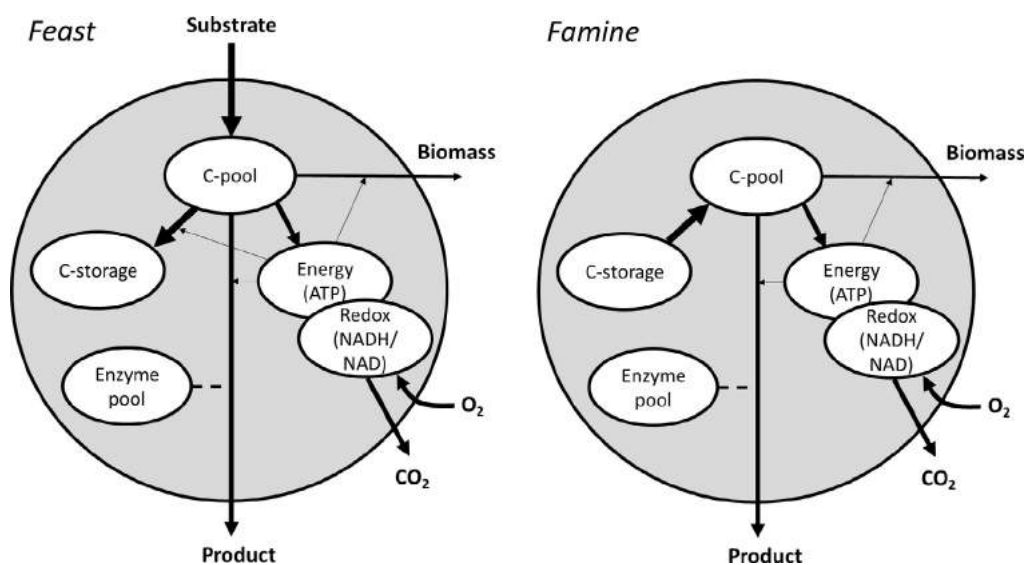
Fig. 9. Key, lumped metabolic pools and fluxes in feast and famine conditions, relevant to large-scale bioreactor operation. When there is sufficient substrate available (left), then the storage pool is filled, while under starvation (right) it is utilized to keep the metabolism going, until depletion of the pool. The enzyme pool represents the rate-determining step in the product formation pathway. It's rate could change under prolonged dynamic conditions.
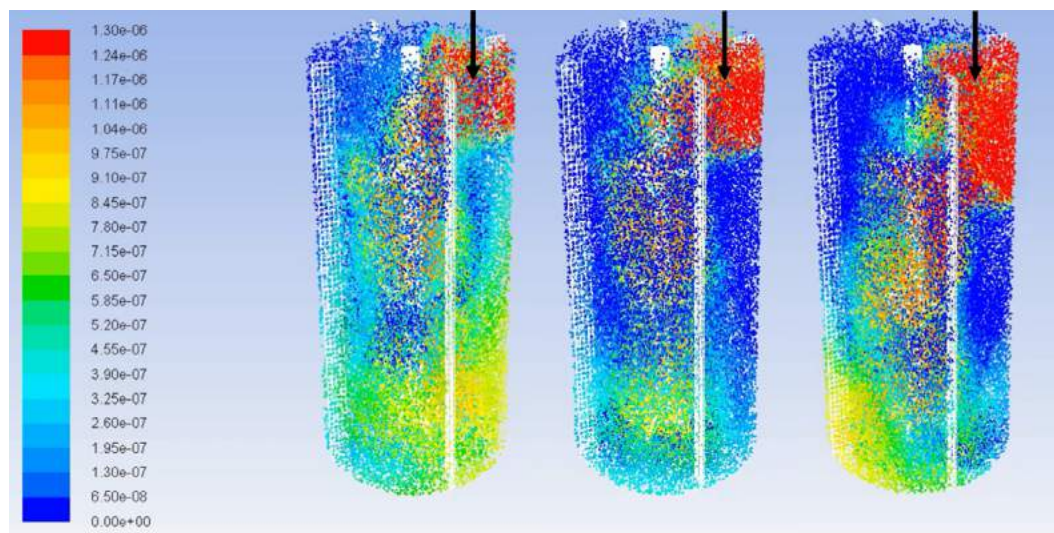
Fig. 10. Three snapshots from a simulation of a large, fed-batch operated bioreactor, with about 10 s in between. Individual microorganisms are colored based on the glucose concentration (mol/l) in their immediate environment. The arrow denotes the feed inlet point. In this sequence, a chaotic flow pattern becomes visible, with rapid downward transport of glucose-rich fluid, and a characteristic radial oscillation of the glucose concentration around the bottom impeller. In this example, the fungus *P. chrysogenum* is applied which has a high affinity for glucose uptake, in the μmolar range (de Jonge et al., 2011). A detailed analysis of the cell life-lines is provided in Haringa et al., 2016.

6. Future perspective

The driving force of the profession of (bio)chemical engineering has been to create and develop new (bio)processes to make the (bio)products wanted by man.

Given the continued pace of computation power increase, it is expected that the role of computation in bioprocess design will tremendously increase in coming years. This follows a clear trend in the global engineering sciences, towards improved predictability based on highly detailed descriptions of real systems, replacing the good but less precise and relatively slow trial-and-error and gross simplification approaches. This is based on deeper understanding of bio/chemo/physical mechanisms and high-resolution structures, and faster computers (Bouhali et al., 2016). Examples are design of airplanes and other transport systems, design and construction of active molecules such as drugs and enzymes, bio/nano functional entities in catalysis and materials, and also in other fields such as architecture. One could view this as a more or less silent revolution in engineering. Also for bioprocess design as well as bioprocess implementation a perspective is now unfolding of fast or even real-time computer simulations, placed next to the plant operations, that reveal the key flow and reaction characteristics, and from this deviations and new opportunities can in principle be identified. It is noted that this does not replace the back-of-the-envelope estimations that will remain vital to a healthy challenge of the designs and computer outputs. One step further than such virtual reality concept, one could consider extension with augmented reality algorithms (Ma and Gausemeier, 2011; Cai et al., 2014; Crandall et al., 2015). This all will speed up process development, and together with the transition to renewable feedstocks, we expect that bioprocesses will make a significant contribution to all three dimensions of sustainability: economic, environmental and social.

At this point it should be noted that training and education of the next generation of biochemical engineers should comprise a good balance of both the design and implementation skills. Levenspiel concluded back in 1988 that the art of conceiving new chemical process concepts was underdeveloped compared to the transformation into reality. Today, for biochemical processes we witness an improved situation, with good examples where students work together in teams trying to define new conversion schemes, and there are graduate schools who have set up specific education programs for bioprocess and bio-product designers. The examples presented in this overview have been extracted from some of these programs in which we have been engaged over the last years, i.e. the massive on-line open course on Industrial Biotechnology (MOOC, 2016), and the postgraduate course program from BioTech Delft (BioTech Delft, 2016). But we are not yet there, as there are grand challenges to tackle and major opportunities to grab towards a sustainable world.

We would further add to this a proposal for the biochemical engineering community to initiate a bioprocess design competition, similar to the successful genetic engineering competition for students (iGEM, 2016). In such design competition, preliminary named iBID, ideal Bioprocess Industry Designs should be generated that can help to overcome significant global sustainability issues. Such contest will stimulate both teachers and the next generation of biochemical engineers to embrace design thinking and benefit from its great potential.

Given this perspective, the scenario that in the coming decades there with be a more than twofold increase of the bio-economy (see Fig. 4), should be realistic. Finding the new landmark bio-products of tomorrow, and putting the associated large-scale bioprocesses into reality, all beginning with the end in mind, then is the grand adventure of biochemical engineering.

Appendix A. Process reactions for conversion of glucose to BDO and BD

A thermodynamic analysis can provide a first estimate of the reaction stoichiometry from glucose to BDO and BD, including also growth and maintenance energy requirements, with only limited information needed (Heijnen, 2010).

Once the product and substrate are known, the first step is to collect the Gibbs energy information for the three main overall reactions in the metabolic network: aerobic glucose catabolism to CO_2 and H_2O for maintenance Gibbs energy generation, formation of biomass, and formation of product. The biomass and product reactions both contain an anabolic part, where biomass and product are formed without involvement of O_2 at the expense of Gibbs energy, and a catabolic part similar to the maintenance reaction, where the Gibbs energy is generated. The overall process reaction is finally found by adding up the five sub-reactions, weighted by their respective rates, which are the maintenance energy formation rate, m_s, the specific growth rate, μ, and the specific product formation rate, q_p. From the case information, the values for $\mu = 0.012$ h^{-1} and $q_p = 0.020$ mol P/mol$_X$ h have been set.

A.1. Gibbs energy information for stoichiometric calculations

- Catabolic reaction for glucose
 $-1C_6H_{12}O_6(aq) - 6O_2(g) + 6CO_2(g) + 6H_2O(l) + 2872$ kJ Gibbs energy
- Maintenance Gibbs energy (Tijhuis et al., 1993)
  $m_G = 4.5 \exp\left[\frac{69\,000}{R}\left(\frac{1}{298} - \frac{1}{T}\right)\right]$ kJ/mol$_x$ h
  R = 8.314 J/mol K
  T = absolute temperature of broth
- Gibbs energy needed in the biomass reaction (Heijnen and van Dijken, 1992)
  $a_G = 200 + 18(6-c)^{1.8} + \exp[((3.8 - \gamma/c)^2)^{0.16} \cdot (3.6 + 0.4c)]$ kJ/mol$_x$
  γ = degree of reduction of 1 molecule C-source
  c = number of carbon atoms in 1 molecule C-source
- Gibbs energy needed for making 1 mol product in the product reaction (Canelas et al., 2011)
  – Active export of product requires 57 kJ/mol product
  – Each irreversible reaction in the pathway (substrate → product) requires a thermodynamic driving force of 35 kJ/reaction. For the BDO pathway there are 9 irreversible reactions, while for BD there are 12
  – The conversion of catabolic energy into biological useful energy occurs with 35% efficiency

A.2. Maintenance reaction rate

The maintenance Gibbs energy requirement follows from the thermodynamic correlation

$$m_G = 4.5 \exp\left[\frac{69\,000}{8.314}\left(\frac{1}{298} - \frac{1}{308}\right)\right] = 11.11 \text{ kJ/mol}_x \text{ h}$$

The maintenance energy is obtained from the catabolic reaction for glucose

$$-1C_6H_{12}O_6(aq) - 6O_2(g) + 6CO_2(g) + 6H_2O(l)$$
$$+ 2872 \text{ kJ Gibbs energy} + 2700 \text{ kJ heat}$$
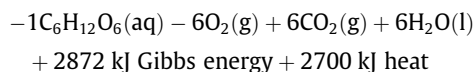
This gives

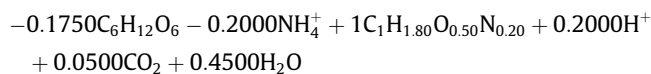$$m_s = -11.11/2872 = -0.00387 \text{ mol glucose/mol}_x \text{ h}$$

It also follows

$$m_o = 6 \, m_s = -0.02322 \text{ mol } O_2/\text{mol}_x \text{ h}$$
$$m_c = -6 \, m_s = 0.02322 \text{ mol } CO_2/\text{mol}_x \text{ h}$$
$$m_w = -6 \, m_s = 0.02322 \text{ mol } H_2O/\text{mol}_x \text{ h}$$

The produced heat is calculated using 450 kJ heat/mol O_2.

A.3. The biomass reaction
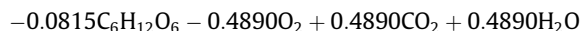
The anabolic part of the biomass reaction reads

$$-0.1750C_6H_{12}O_6 - 0.2000NH_4^+ + 1C_1H_{1.80}O_{0.50}N_{0.20} + 0.2000H^+$$
$$+ 0.0500CO_2 + 0.4500H_2O$$

The Gibbs energy needed to make biomass follows from the correlation for a_G, using $\gamma = 24$ and $C = 6$ for glucose

$$a_G = 200 + 0 + \exp(0.59 \cdot 6) = 200 + 34 = 234 \text{ kJ/mol}_x$$
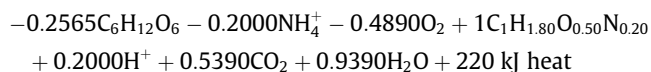
The catabolic energy gain for glucose is 2872 kJ/mol glucose, therefore the catabolic glucose part follows as

$$234/2872[-1C_6H_{12}O_6 - 6O_2 + 6CO_2 + 6H_2O]$$

Or

$$-0.0815C_6H_{12}O_6 - 0.4890O_2 + 0.4890CO_2 + 0.4890H_2O$$

The biomass reaction from glucose is obtained from summing the anabolic and catabolic parts

$$-0.2565C_6H_{12}O_6 - 0.2000NH_4^+ - 0.4890O_2 + 1C_1H_{1.80}O_{0.50}N_{0.20}$$
$$+ 0.2000H^+ + 0.5390CO_2 + 0.9390H_2O + 220 \text{ kJ heat}$$

A.4. The product reaction

A.4.1. BDO from glucose
The anabolic part of the product reaction follows as

$$-0.91666C_6H_{12}O_6(aq) + 1C_4H_{10}O_2(aq) + 1.50CO_2(g)$$
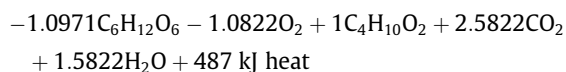$$+ 0.50H_2O(l) + 191 \text{ kJ Gibbs energy}$$
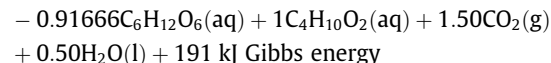
Note that this anaerobic sub-reaction produces 191 kJ/mol BDO.
For the catabolic part we need to know the required biological energy input. From the case information this follows as $-191 + 9 \cdot 35 + 57 = 181$ kJ/mol BDO. With 35% efficiency this requires catabolically produced Gibbs energy of 181/0.35 = 518 kJ/mol BDO.
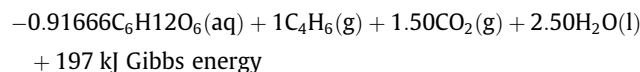This is obtained by catabolism of glucose for 1 mol BDO

$$518/2872[-1C_6H_{12}O_6 - 6O_2 + 6CO_2 + 6H_2O]$$

Adding up catabolism and anabolism gives the product reaction of BDO on glucose

$$-1.0971C_6H_{12}O_6 - 1.0822O_2 + 1C_4H_{10}O_2 + 2.5822CO_2$$
$$+ 1.5822H_2O + 487 \text{ kJ heat}$$

A.4.2. BD from glucose
The anabolic part follows as

$$-0.91666C_6H12O_6(aq) + 1C_4H_6(g) + 1.50CO_2(g) + 2.50H_2O(l)$$
$$+ 197 \text{ kJ Gibbs energy}$$

For the catabolic part we need to know the required biological energy input. From the case information this follows as
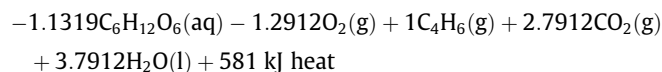
$$-197 + 12 \cdot 35 + 0 = 223 \text{ kJ/mol BD}$$

With 35% efficiency this requires a catabolic Gibbs energy production of 223/0.35 = 638 kJ/mol BD.
This energy is provided from catabolism of glucose

$$638/2872[-1C_6H_{12}O_6 - 6O_2 + 6CO_2 + 6H_2O]$$

Adding up of the anabolic and catabolic parts results in the product reaction for BD from glucose

$$-1.1319C_6H_{12}O_6(aq) - 1.2912O_2(g) + 1C_4H_6(g) + 2.7912CO_2(g)$$
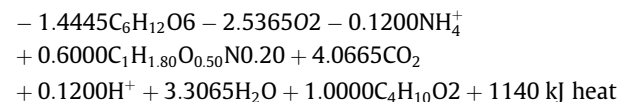$$+ 3.7912H_2O(l) + 581 \text{ kJ heat}$$

A.5. The process reactions

The process reactions can now be obtained from the known biomass specific rates ($m_s = -0.00387$ mol glucose/mol$_x$ h, $\mu = 0.012$ h^{-1}, $q_p = 0.020$ mol product/mol$_x$ h), and the reaction stoichiometries for the maintenance, biomass and product reactions.

A.5.1. BDO from glucose

$$q_s = -0.2565(0.012) - 1.0971(0.020) - 0.00387 = -0.02889$$
$$q_{O2} = -0.4890(0.012) - 1.0822(0.020) - 0.02322 = -0.05073$$
$$q_{CO2} = +0.5390(0.012) + 2.5822(0.020) + 0.02322 = +0.081133$$
$$q_w = +0.9390(0.012) + 1.5822(0.020) + 0.02322 = +0.06613$$
$$q_h = +0.2000(0.012) + 0(0.020) + 0 = +0.0024$$
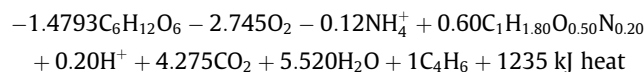$$q_n = -0.2000(0.012) + 0(0.020) + 0 = -0.0024$$

In the process reaction the coefficients need to be normalized with q_p. The above q-values divided by q_p give the process reaction

$$-1.4445C_6H_{12}O6 - 2.5365O2 - 0.1200NH_4^+$$
$$+ 0.6000C_1H_{1.80}O_{0.50}N0.20 + 4.0665CO_2$$
$$+ 0.1200H^+ + 3.3065H_2O + 1.0000C_4H_{10}O2 + 1140 \text{ kJ heat}$$

A.5.2. BD from glucose

$$q_s = -0.2565(0.012) - 1.1319(0.020) - 0.00387 = -0.02959$$
$$q_{O2} = -0.4890(0.012) - 1.2912(0.020) - 0.02322 = -0.0549$$
$$q_{CO2} = +0.5390(0.012) + 2.7912(0.020) + 0.02322 = +0.0855$$
$$q_w = +0.9390(0.012) + 3.7912(0.020) + 0.02322 = +0.1104$$
$$q_h = +0.2000(0.012) + 0(0.020) + 0 = +0.0024$$
$$q_n = -0.2000(0.012) + 0(0.020) + 0 = -0.0024$$

The process reaction follows as

$$-1.4793C_6H_{12}O_6 - 2.745O_2 - 0.12NH_4^+ + 0.60C_1H_{1.80}O_{0.50}N_{0.20}$$
$$+ 0.20H^+ + 4.275CO_2 + 5.520H_2O + 1C_4H_6 + 1235 \text{ kJ heat}$$

Appendix B. Conversion rates and concentrations for the BDO and BD processes

Gas flows will be calculated in mol/h and their composition in mol fractions. Liquid flows will be calculated in kg/h and their composition in mol/kg. The required results can be obtained from combining the case inputs and assumptions:

- R_p = 70 000 mol product/h
- Process reaction stoichiometries obtained derived in Appendix A
- Steady State balances where the R_i terms are known from R_p and the process reaction
- Composition of the dry air used for sparging
  $y_{o,in}$ = 0.21, $y_{c,in}$ = 0, $y_{w,in}$ = 0
- Composition of the substrate feed and titrant liquids: concentrated glucose solution (720 g/kg = 4 mol/kg) is used as feed and KOH (8 mol/kg) is used as titrant. The N-source is $(NH_4)_2SO_4$ which is added to the glucose feed solution. The feed temperature is 50 °C (after sterilisation with heat recovery)
- The mol fraction CO_2 in the off gas (CO_2 inhibition at 0.05 bar partial pressure)
- The concentrations required by the organism: c_s = 0.05 · 10^{-3} mol/kg and c_{NH4+} = 5 · 10^{-3} mol/kg

For the BDO case detailed calculations will be provided. For the BD case the same approach is used and only the final result is given in Fig. 7.

We will start with the gaseous flows because the water evaporation is an important issue.

- $F_{N,out}$ (mol gas/h)
  The gas outflow follows from the CO_2 inhibition. We assume a top pressure P_t = 1 bar, which gives $y_{c,out}$ = 0.05 from $P_{c,inhibit}$ = 0.05 bar. The process reaction gives R_c = 70 000 · 4.0665 molCO_2/h. From the gas phase CO_2 balance it follows $F_{N,out}$ = 70 000 · 4.0665/0.05 = 5 963 100 mol/h

- $y_{w,out}$ and water evaporation rate
  At temperature (T = 308 K) the water partial pressure is P_w = 0.0584 bar
  Note this holds for pure water but as shown below the broth is highly concentrated (BDO ≈ 450 g/kg, so the P_w may in reality be lower). Later one could consider this 2nd order effect.
  The water evaporation rate through the off gas (P_t = 1 bar, y_w = 0.0584) follows from the gas phase water balance as
  $F_{N,out} \cdot y_w$ = 5 693 100 · 0.0584 = 332 477 molH_2O/h = 5985 kg water/h

- $F_{N,in}$ (mol/h)
  The gas inflow follows from a total mol balance in the gas phase (mol/h)
  $F_{N,in}$ + 70 000 · 4.0665(CO_2) + 332 477(H_2O) = $F_{N,out}$ + 70 000 · 2.5365(O_2) which gives $F_{N,in}$ = 5 253 523 mol/h
  Note that $F_{N,out}$ > $F_{N,in}$ due to water evaporation and CO_2 production being much larger than O_2 consumption

- $y_{O2,out}$
  For the composition of the off gas, the values for $y_{c,out}$ (CO_2) and $y_{w,out}$ (H_2O) are known. What is missing is mol fraction O_2, $y_{O2,out}$. This unknown is present in the gas phase O_2 balance, which follows using the process reaction:
  5 253 523 · 0.21 − 70 000 · 2.5365 = 5 693 100 · $y_{O2,out}$ leading to $y_{O2,out}$ = 0.1626
  Check gas phase N_2-balance
  In: 5 253 523 · 0.79 = 4 150 283 molN_2/h
  Out: 5 693 100 (1 − 0.1626 − 0.0584 − 0.050) = 4 150 270 mol/h, which leads to a closed gas phase N_2-balance (considering round off errors)

- $F_{m,in}$ liquid feed (kg/h)
  The required $(NH_4)_2SO_4$ in the feed solution follows from the broth NH_4^+ balance and the process reaction and is 70 000 · 0.12/2 = 4200 mol/h (or 554 kg/h). Neglecting the 2.5 mol $(NH_4)_2SO_4$/m^3 in the broth outflow (to be checked later) this mass flow of $(NH_4)_2SO_4$ must be present in the aqueous glucose feed solution.
  The consumed glucose follows from R_p = 70 000 mol P/h and the process reaction as 70 000 · 1.4445 = 101 115 mol glucose/h. When we neglect the glucose present in the broth outflow of 0.05 mol glucose/ton broth, the broth glucose balance requires a glucose 720 g/kg feed solution (4 mol glucose/kg solution) of 101 115/4 = 25 279 kg/h.
  This leads to a combined (glucose + $(NH_4)_2SO_4$) liquid feed mass rate:
  $F_{m,in}$ = 25 279 + 554 = 25 833 kg/h
  The composition follows as
  – $c_{s,in}$ = 101 115/25 833 = 3.9142 mol/kg
  – c_{NH4+} = 8400/25 833 = 0.3252 mol/kg
  – $c_{SO42−}$ = 0.1626 mol/kg
  – c_{H2O} = 15.22 mol/kg

- $F_{m,titr}$ titrant
  The rate of produced H$^+$ is 70 000 · 0.12 = 8400 mol H$^+$/h. The titrant requirement follows from a broth H$^+$ balance as (8 mol OH$^-$/kg)
  $F_{m,titr}$ = 8400/8 = 1050 kg titrant/h

- $F_{m,out}$ broth outflow (kg/h)
  The broth total mass balance gives the broth outflow, using
  – O_2 uptake = 70 000 · 2.5365 · 0.032 = 5682 kg/h
  – CO_2 emission = 70 000 · 4.0665 · 0.044 = 12 525 kg/h
  – Evaporation of water 5985 kg/h
  – The 2 liquid feeds (glucose and titrant)

  $F_{m,out}$ = 25 833 + 1050 + 5682 − 12 525 kg/h − 5985 = 14 055 kg/h

- The broth outflow composition
  In this broth there is biomass, K$^+$, SO_4^{2-}, product at concentrations obtained from
  – K$^+$-balance: c_{K+} = 8400/14 055 = 0.5977 mol/kg
  – SO_4^{2-} balance: $c_{SO42−}$ = 4200/14 055 = 0.2990 mol/kg
  – Biomass balance: c_x = 700 000 · 0.60/14 055 = 2.988 mol/kg
  – Product balance: c_p = 70 000/14 055 = 4.980 mol/kg

  In addition there is present in the broth c_s = 0.05 · 10^{-3} mol/kg and c_{NH4+} = 5 · 10^{-3} mol/kg. The neglected SO_4^{2-} in the outflow (associated with NH_4^+, 2.5 · 10^{-3} mol/kg) is only 0.8% of the previously calculated SO_4^{2-}-concentration and is therefore indeed negligible. The neglected glucose outflow is 0.7 mol glucose/h, which is 0.007% of the glucose feed. It is obvious that water evaporation and C-loss has a significant impact: broth mass outflow is 40% less than the mass inflow.
  This shows that we need accurate water vapour pressure data for the highly concentrated broth (448 g BDO/kg). It is expected that the real water vapour pressure is considerably lower, leading to less evaporation and lower BDO concentrations.

- F_Q: heat to be removed in kJ/s
  The heat balance gives the heat flow that needs to be transported out of the vessel:
  biological heat production = 70 000 · 1140 = 79 800 000 kJ/h
  Heat input from mechanical energy for O_2-transfer = 70 000 · 2.5365 · 58 = 10 298 190 kJ/h

Heat from warm feed $(50\,°C) = 25\,833 \cdot 4.18 \cdot (50-35) = 1\,619\,729$ kJ/h

Water evaporation $= -14\,296\,511$ kJ/h

It is clear that the cooling from water evaporation and the heat from mechanical energy input for O_2-transfer is significant (but this is likely too high because there is probably less water evaporation). The balance shows the total heat load

$F_Q = 77\,421\,408$ kJ/h $= 21\,506$ kJ/s

- The broth mass M follows from R_p $(=70000$ $mol_p/h)$ and q_p $(=0.20$ mol_p/h per mol biomass) which gives $N_x = R_p/q_p = 3.50 \cdot 10^6$ mol_x.

  Because $c_x = 2.988$ mol_x/m^3 there is $M = 3.50 \cdot 10^6/2.988 = 1\,171\,352$ kg broth in the fermentor

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