Bioreactor technology for sustainable production of plant cell- and tissue-derived products

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Abstract:

The successful cultivation of plant cell and tissue cultures for the production of valuable chemical components requires the selection of an appropriate bioreactor. Selection criteria are determined based on a number of factors that are intrinsic to particular plant cell or tissue cultures and are influenced by the process objectives. Due to the specific properties of plant cell and tissue cultures, bioreactor systems may differ significantly from those used for microorganism or animal cell cultures. Furthermore, the differences from one plant culture to another can be immense; it is obvious that the optimal bioreactor system for a plant suspension cell culture is different to one for a plant tissue culture in many ways.

General considerations are presented, and based on these key points, selection criteria are used to establish a "bioreactor chooser" tool. The particular details of the most relevant bioreactor types for plant cell and tissue cultures are listed and described.

To produce valuable products, the process also needs to be scaled up to an economically justifiable size, which is usually done either by scaling up the size of the bioreactor itself or by bioreactor parallelisation. Therefore, the most significant influencing factors are also discussed.

Abbreviations

2G12	Human monoclonal antibody 2G12
DAF-Fc	Decay-accelerating factor-fragment crystallizable region
DPP4-Fc	Dipeptidyl-Peptidase 4 fragment crystallizable region
FDA	Federal Drug Administration
GAD65	Glutamate decarboxylase 65
GMP	Good manufacturing practice
HA	Hemagglutinin
HCPS	Hantavirus cardiopulmonary syndrome,
hG-CSF	Granulocyte-colony stimulating factor
hGM-CSF	Human granulocyte-macrophage colony-stimulating factor
ICAM-1-IgA2	Intercellular adhesion molecule 1 Immunoglobulin A2
ICH	International Conference on Harmonisation
IL-12	Interleukin 12
Mers	Middle East respiratory syndrome
OUR	Oxygen uptake rate
QbD	Quality by design
RITA	Récipient à immersion temporaire automatique

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1 Introduction

Plants are an essential component in human diets as they produce carbohydrates, lipids (fatty acids), proteins (amino acids), and vitamins (e.g. ascorbic acid), as well as storing macro (e.g. magnesium) and trace elements (iron). The relevance of plant cell and tissue cultures has been drawing more and more attention in the biotechnological industry [1–3] over the past decade, with the production of secondary metabolites and recombinant proteins being of particular interest.

Besides plants being fundamental components in animal, mammalian, and human food chains since prehistoric time, mankind has also used plants to cure illnesses and injuries, to dye cloths and for spiritual purposes. Knowledge has been passed down and enhanced from generation to generation. Most of the positive effects of herbs and other plant material are based on complex chemical compounds, often referred to as secondary metabolites. These secondary metabolites are usually small but complex molecules, which are in many cases impossible or prohibitively expensive to synthesize chemically [4, 5]. Based on their metabolic pathways and their biogenetic precursors, they can be classified into three groups: terpenoids (e.g. paclitaxel, ginsenosides), alkaloids (e.g. morphine) and phenolics (e.g. shikonin, rosmarinic acid) [6]. Today, secondary metabolites are used directly or as precursors for the production of pharmaceuticals, cosmetics, fragrances, flavours, dyes, insecticides and much more [7–9]. The extraction of secondary metabolites from plants, which were traditionally grown in fields, is still the main production method for these substances [1]. However, there are a number of disadvantages associated with traditional farming, one of which is the excessive variation of environmental conditions over time and region, which leads to unpredictable differences in the quality and quantity of the raw materials.

Many secondary metabolites are produced in plants that are not suited to agricultural production or can be hard to grow outside their local ecosystems [10], and thus the extensive exploitation of these plants could potentially lead to their extinction. As a result, it is not surprising that around one fifth of the 50,000 medical plants that are used today are on the list of threatened species [1]. Therefore, plant cell and tissue cultures grown in bioreactors offer an eco-sustainable alternative. Furthermore, the metabolic pathways of secondary metabolites often contain many branches, and thus the transfer of the genetic information to common biotechnological production organisms often fails to deliver the desired results [4]. As a result, plant cell and tissue cultures are believed to represent an appropriate method that addresses the main drawbacks of traditional farming of herbs and other

useful plants, and avoids the problems associated with extracting products from protected wild plants.

A famous example for a pharmaceutically used secondary metabolite derived from plant cell cultures is paclitaxel, an anti-cancer drug. Previously produced by harvesting the bark from *Taxus sp.* trees, today, the large scale production of paclitaxel is performed in stirred stainless steel bioreactors up to a culture volume of 75 cubic metres using plant suspension cell cultures of *Taxus sp.* by Phyton Biotech from Ahrensburg, Germany [11, 12]. The Swiss cosmetics company Mibelle uses two different types of bioreactors for the production of apple suspension cells for their PhytoCellTech™ product. This involves using a single-use vibrating disk bioreactor at a scale of 50 L and numerous single-use wave-mixed bag bioreactors to produce biomass for the cosmetic industry [11, 13, 14].

Recently, more and more attention has been paid to how plants can be used as hosts for the production of therapeutic proteins [15–17]. Currently, one plant-based therapeutic protein is approved for human treatments: the recombinant glucocerebrosidase known as Elelyso®, which is used to treat Gaucher disease and has been produced by the Israeli company Protalix and Pfizer since its registration and approval by the Federal Drug Administration (FDA) in 2011 [18]. It is produced with a carrot based suspension cell line in single-use pneumatically driven bioreactors at a scale of 400 L [16, 18, 19]. In order to scale-up to production quantities, many bioreactors are used in parallel. A poultry vaccine for the Newcastle disease virus has been by approved by US regulatories for veterinary use only in 2006 [15], which is produced with tobacco cells. The cells are lysed and injected subcutaneously into chickens. It is the only veterinary vaccine, however, it was never commercialized [20].

Recently, many more different modern biopharmaceutical compounds [3, 21–23] have been investigated for plant production processes: Zmapp, a vaccine against ebola [24], GAD65, a key autoantigen in type 1 diabetes, Norwalk virus-like particles, the monoclonal antibody 2G12 for utilization against HIV [25], HA vaccine against influenza [26], personalized medicines like a vaccine against non-Hodgkin's lymphoma for individual patients [27], CaroRx targeting cavity-causing bacteria *Streptococcus mutans* [28] and many more. Furthermore, there have also been descriptions of how immunoadhesins, antibody-like, chimeric molecules, which possess the functional domain of a binding protein (a receptor, ligand or cell-adhesion molecule) with immunoglobulin constant domains [29], like ICAM-1-IgA2 (against Human rhinoviruses), DPP4-Fc (against Middle East respiratory syndrome,

MERS) and DAF-Fc (against hantavirus cardiopulmonary syndrome, HCPS) have been successfully produced in tobacco plants [30].

Besides their application for pharmaceuticals, many recombinant proteins are available for cosmetic applications. Growth factors (e.g. hGM-CSF, IL-12, hG-CSF) are produced in rice suspension cells for cosmetic application [31].

Producing pharmaceutical compounds requires strict compliance with rules laid down by the International Conference on Harmonisation (ICH) and its participating government authorities from US, Europe and Japan [32]. In order to comply with these rules, undefined conditions that exist in agriculturally produced plant cells need to be avoided. Besides the ease of complying with good manufacturing practice (GMP) regulations thanks to the quality by design (QbD) approach when using bioreactor based production processes with plant cell or tissue cultures, shorter production cycles (days to weeks) can also be expected in comparison to using whole plants that involve a production cycle of months. Furthermore, the complete elimination of environmental variations leads to improved consistency between batches, which is crucial for gaining official acceptance. The avoidance of labour intensive greenhouse or field production of whole plants reduces costs: not only in upstream processing, but also in downstream processing, in particular in the case of products which are secreted into the medium [33]. Obviously, the safety of the process with regards to product contamination with endotoxins and mycotoxins and, of no less importance, with regards to environmental contamination with artificial, genetically modified plants is tremendously enhanced when operating in a closed bioreactor system [23, 34].

2 Plant cell culture demands

The scaling up potential of a bioreactor is one of the main aspects that should be considered very carefully (see Fig. 1). While it is reported that stirred devices can be scaled up to seventy-five cubic metres [12], other bioreactor types available on the market are limited to several hundred litres (e.g. wave-mixed bioreactors) [35]. Furthermore, the footprint of a bioreactor plays a crucial role, which is a further disadvantage of wave-mixed and orbitally shaken bioreactors. However, as energy input represents a critical limitation for suspension and tissue cultures in particular, alternative agitated systems are important for these culture types. Gas exchange capacity is another relevant parameter for optimal cultivation results and depends

heavily on the energy input method and magnitude. Finally, special cultivation requirements, such as constant illumination for photoauto- and photomixtrophic cultures, further increase complexity, since it is hard to dose light as a non-mixable reactant.

One of the most important functions of a bioreactor is adequately dosing the energy that is input into the culture broth. Sufficient mixing (to prevent nutrient limitations or the accumulation of hazardous by-products), gas-exchange (to provide enough oxygen/carbon dioxide for respiration), and dispersion (to prevent sedimentation, especially in suspension cultures) must be ensured. However, excessive energy input may harm the tissue (e.g. the root networks and the "hairy" extensions of hairy roots) [36, 37], decrease the embryonic potential of embryonic cultures or damage the cells, and thus leads to reduced biomass concentrations and product titres. Finally, it is vital to remember that mechanical stress may influence secondary metabolite production in two ways: either as an increasing elicitor of product formation or as an inhibiting disturbance, which varies depending on the amount of energy, the distribution method and the plant species.

Mixing is heavily influenced by the rheological properties of the liquid. Plant cell suspensions exhibit water-like fluid characteristics at the inoculum stage. An increase in viscosity and often non-Newtonian rheology is inherent to most plant cell suspensions [38].

Guaranteeing sufficient aeration is another crucial factor in bioreactor design. In heterotrophic plant cultures, the supply of oxygen may limit growth. However, high aeration rates also induce shear stress and lead to increased foaming and evaporation. The average oxygen demand of plant cells is comparably low (compared to microbial processes). Typical values for the oxygen uptake rate (OUR) of plant suspension cells are in the range of 5 to 10 $mmol_{O2}$ L^{-1} h^{-1}, which is comparable to animal cells with an OUR value of approximately 0.05 to 10 $mmol_{O2}$ L^{-1} h^{-1}, but much lower than microbial cell values of 10 to 90 $mmol_{O2}$ L^{-1} h^{-1} [22]. For slow growing suspension cells or tissues (e.g. root cultures) in particular, the importance of aeration is eclipsed by the potential damage that can result from shear forces. In addition, high aeration provokes increased foaming and thus reduces surface gas exchange and increases the risk of clogged sterile filters.

To deal with the above mentioned issue, the first step is to select the bioreactor type with the most suitable specific oxygen transfer coefficient (k_La) to specific power input (P/V) ratio. Afterwards, the bioreactor design and operating method may be

adapted, e.g. by employing different spargers or by supplying oxygen enriched inlet air. The utilization of antifoam solutions must be carefully considered, since they may reduce biomass growth, product quality, and oxygen transfer, and thus lead to even higher aeration rates [39–41].

Photoautotrophic and photomixotrophic cultures with suspension cells or tissue require light as an energy source for their metabolism. In order to provide light with a certain photon density to the plant cells or tissue, it is crucial to keep the layer thickness of the culture as thin as possible. Adequate distribution of illumination is quite difficult, since light is not mixable. Furthermore, higher biomass concentrations drastically increase the absorption properties of the culture broth, leading to reduced illumination as penetration depth rises. As a result, different cells are exposed to different amounts of light and the specific growth rate may vary in different regions of the bioreactor [42]. However, increased illumination does not increase growth rates in equal measures, and light inhibition may occur [43]. For light dependent cultures, flat-panel reactors seem to be the most promising way to overcome these obstacles.

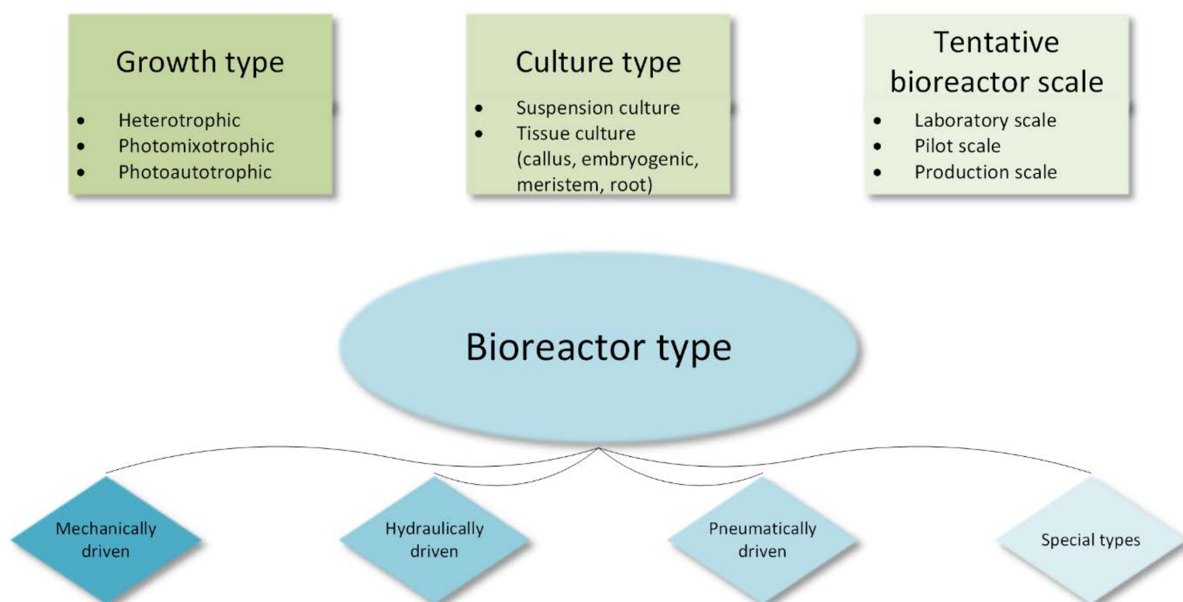

Fig. 1: Factors that influence the bioreactor decision process. The growth type, the culture type and the final indented bioreactor scale represent key factors of the decision process.

3 Bioreactor selection process

The selection of a suitable bioreactor is anything but easy. As a rule of thumb, large scale suspension cell cultures are most profitable in stirred tanks. Cells with a high sensitivity to mechanical stress may be cultivated in orbitally or wave mixed bags, however scalability is limited and parallelisation may be required. A simplified selection scheme is depicted in Fig. 2. More detailed information can be found in the following subsections.

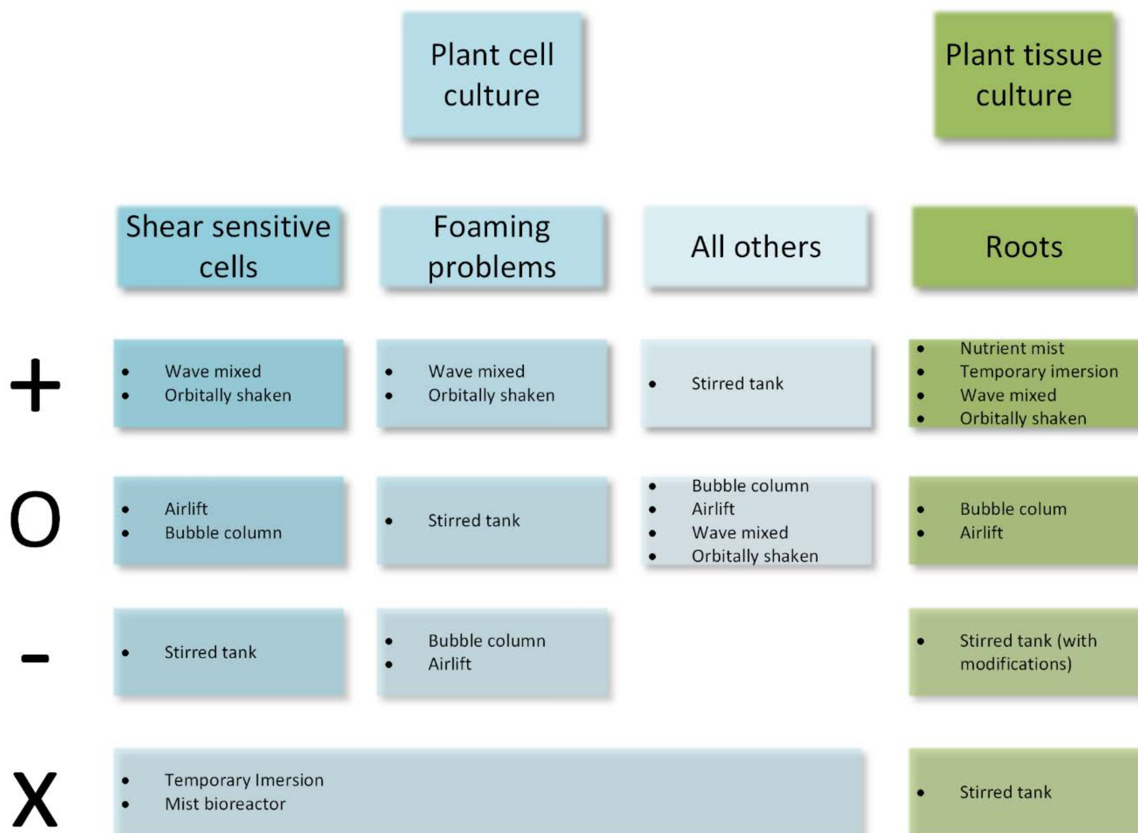

Fig. 2: Bioreactor chooser, based on culture type and limiting factors

3.1 Stirred tank reactor

The classical stirred tank bioreactor (Fig. 3 A) is widespread in biotechnology and has multiple uses [1]. Agitation and mixing is performed by one or several stirrers [44]. Frequently used stirrers for plant cell cultures are marine impellers and pinched-blade turbines, which create axial fluid flow patterns at a comparably low tip speed (up to 2.5 m s^{-1}) [45]. An overview of suitable impellers is provided by Eibl and Eibl [45] and Doran [4].

Due to variations in vessel geometry and impellers [46], stirred bioreactors are highly adaptable [47]. Furthermore, the controllability of process parameters like pH, temperature and oxygen concentration in stirred tanks is superior to all other bioreactors [48], making stirred bioreactors extremely well-suited to the cultivation of robust suspension cells. Unfortunately, all these benefits cannot outweigh the fact that the moving impeller mechanically damages the tissue and thus leads to reduced growth or death. Therefore, there are two reasonable options for growing plant cell tissues: Either a stirred tank reactor can be adapted (e.g. with a constructive separation between agitation and growing compartments [49]) to the requirements of plant cell tissue cultures or bioreactors can be used that are agitated in a different way.

3.2 Bubble column reactor

A bubble column bioreactor (Fig. 3 D) can be simpler than nearly any other bioreactor, consisting of a cylindrical vessel and a bottom-mounted sparger. Mixing and agitation are both performed by the rising air bubbles without further mechanical energy input [23, 50]. The lack of moving parts reduces the risk of contamination. Due to their homogeneous and low power input, bubble columns are well suited to plant tissue cultures. However, the potential occurrence of shortcuts, mass transfer limitations, foaming and floatation limit their application [48], especially for high density suspension cultures [23]. Bubble columns can be further improved by incorporating several stages or static mixers.

3.3 Airlift reactor

Another pneumatically agitated bioreactor is the airlift reactor (Fig. 3 E). It resembles a bubble column but includes the addition of a draft tube (used for the creation of an internal or external loop) [51]. As a result of this circulation, the oxygen transfer is higher and the mixing times and shear forces are lower than in a comparable bubble column reactor [1, 45]. Different modifications exist, e.g. a combination with a perfusion system for high cell densities proposed by Wie Wen Su et al. [52] or an

illuminated system for photoautotrophic cultivation of plant cell suspension cultures proposed by Fischer and Alfermann [53].

3.4 Orbitally shaken bioreactors

The group of orbitally shaken bioreactors (Fig. 3 B) consists of several geometrical dissimilar vessels from millilitre to cubic metre scales. Centrifuge tubes and Erlenmeyer, Fernbach and Thomson Optimum Growth™ shake flasks are used for screening purposes (e.g. media optimization) and inoculum production at the laboratory scale and have been quite well examined [13, 54, 55]. The next step in a scale up process may include orbitally shaken bag bioreactors (which are similarly shaped to wave mixed bags). Due to the low shear forces combined with moderate aeration and good mixing, bag bioreactors seem promising for plant cell and tissue cultures [56]. Large scale cultivations utilising orbitally shaken reactors containing *Nicotiana tabacum* suspension cells for manufacturing recombinant antibodies have shown that volumes can be increased up to a small scale production size of 200 L [57, 58].

Since there are no moving parts inside the reactor, energy is provided via the vessel walls. Aeration may be passive (e.g. for shake flasks) or active (e.g. for bag bioreactors). In the case of bag bioreactors, the aeration also ensures that the bags are fully inflated. The straightforward design and the availability of disposable orbitally shaken vessels make it easier to conform to GMP and reduce contamination risks. Despite their lower aeration and mixing rates and inferior controllability in comparison to a stirred tank, orbitally shaken bioreactors have the great advantage of being able to be used for cell suspension and tissue cultures without needing to be adapted.

An intermediary between orbitally shaken and wave-mixed systems is the travelling wave bioreactor, which is orbitally agitated to produce a travelling wave [59–61].

3.5 Wave-mixed bag bioreactor

As the name implies, energy dissipation, and hence mixing and oxygen transfer in the wave-mixed bioreactors (Fig. 3 C) are realised by inducing waves. Adjustments may be made by changing the bag itself (e.g. size and length to width to height ratio), the operating conditions (e.g. rocking rate and angle, and aeration rate) and the cultivation conditions (e.g. working volume) [35, 62, 63]. Oxygen mass transfer is bubble-free due to gas exchange on the surface, lowering foaming tendencies. Usually, these types of bioreactors are disposable and are delivered pre-sterilized.

Hence, the cross-contamination risk is relatively low and it is comparatively easy to perform cultivations that conform to GMP. However, for academic research or for cultivations that do not conform to GMP, re-usable options may be promising, e.g. the polycarbonate bioreactor proposed by Scholz and Suppmann [64].

One of the main advantages of the wave-mixed bioreactors is its applicability for plant cell suspensions and tissue cultures. Shear sensitive or foaming suspensions as well as fully and partially submersed root cultures can be cultivated with low rocking rates in the wave-mixed bioreactors. However, not all commercially available bag bioreactors are suitable for plant suspension cultivations, since ports made for animal cells or microbial cultivations are not wide enough, and thus tubes may become blocked. For this reason, plant culture bags have enlarged sampling and inoculation ports. More detailed information about disposable wave-mixed bioreactors can be found elsewhere [35, 65, 66].

3.6 Nutrient mist and nutrient sprinkle reactors

The nutrient mist and the nutrient sprinkle bioreactors (Fig. 3 F) are both designed to satisfy the needs of hairy and adventitious root cultures. In both systems, the roots are immobilized on a mesh, a steel matrix or a porous structure. The medium is distributed as an aerosol (mist reactor) or as small droplets (sprinkle reactor) from the top of the bioreactor [67]. It is then recirculated and may be stored in a reservoir tank. This class of bioreactors is characterised by extremely low mechanical stress and high gas mass transfer rates [68, 69]. However, the designated plant tissues should be adapted to non-submersed growth. Furthermore, the absence of shear forces may lead to reduced growth and product formation rates, as mechanical stress may act as an elicitor of secondary metabolites. A comparison of nutrient mist and sprinkle bioreactors with other systems has been done by Mishra and Ranjan [51] and Nuutila et al [70].

3.7 Temporary immersion system

Temporary immersion systems (Fig. 3 G) are another type of bioreactor focused on cell tissue cultures. The operating principle is the alternation of submerged and non-submerged periods, equivalent to low and high tide. Adjusting these tidal times is a simple but effective method for controlling metabolite and gas exchange, while keeping the mechanical stress low. Some common implementations of the temporary immersion approach are the twin-flask system, the ebb-and-flow system, the RITA® system and the thermos-photo-bioreactor [71–73]. A broad review of temporary immersion systems has been provided by Georgiev et al [74].

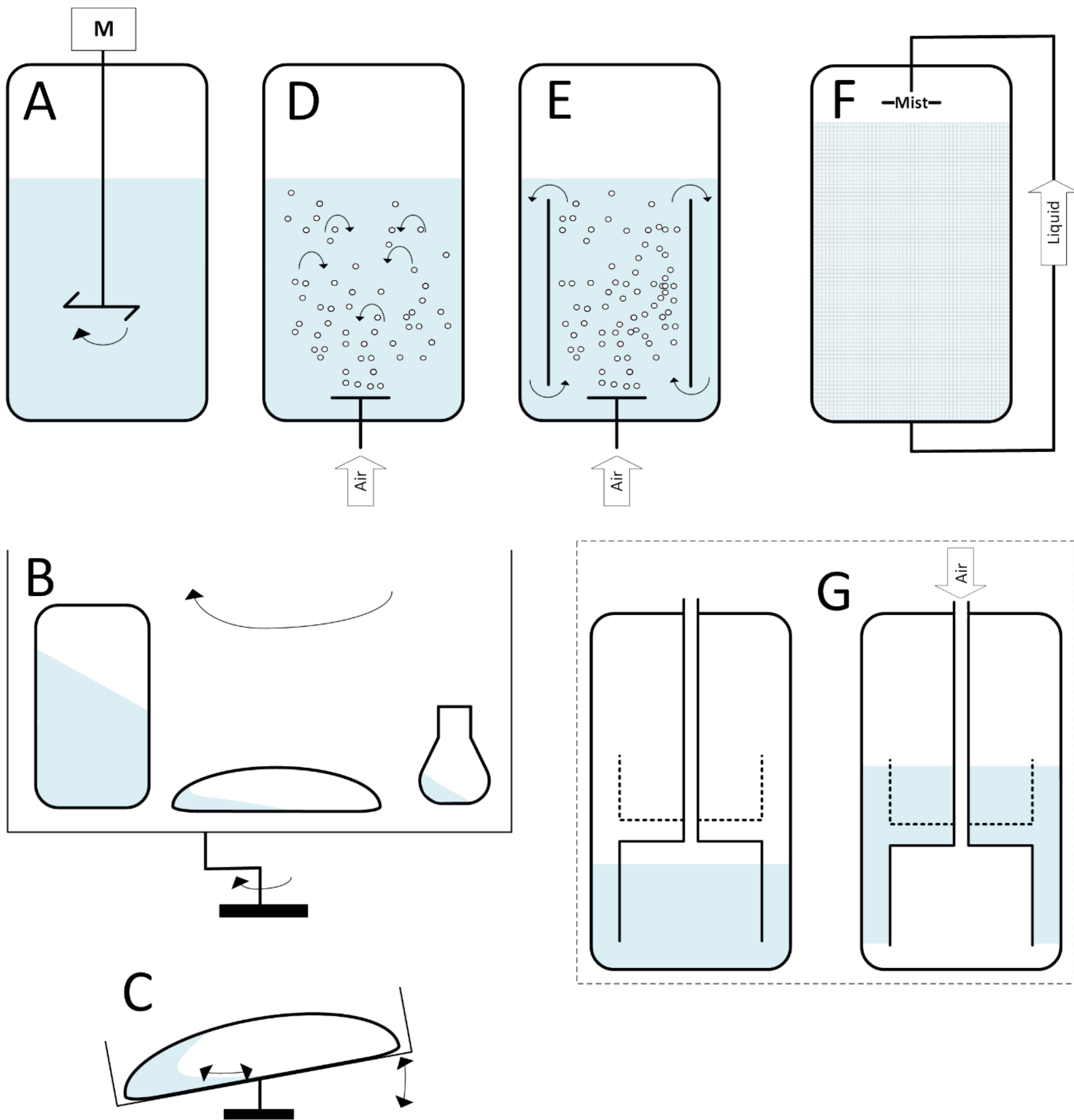

Fig. 3: Mixing principles of the most important bioreactor types (A: Stirred bioreactor, B: Orbitally shaken bioreactors with different vessel shapes, C: Wave-mixed bioreactor, D: Bubble column, E: Airlift bioreactor, F: Mist bioreactor and G: Temporary immersion system)

4 Engineering and scale-up considerations

4.1 Plant cell suspension rheology

In many cases, the low productivity of secondary metabolites by plant suspension cells requires a scale-up in bioreactor technology of tens of cubic meters in order to achieve an economical production process. In general, a correlation of fixed costs per kg product to bioreactor volume to the power of minus two thirds is assumed, meaning products become about five times cheaper with a ten-fold increase in production volume [75]. Currently, the scale-up of agitated vessels for plant suspension cell cultures generally follows particular rules that date back several decades and are mostly based on a certain level of geometric similarity between the vessels [76]. A lack of geometric similarity often leads to scale-up approaches based on a number of engineering parameters, such as the volumetric oxygen mass transfer coefficient $k_L a$ [77, 78], mixing time [79, 80] or power input [81].

One of the most important engineering parameters for dimensioning and scaling-up of bioreactors and for the design of process parameters is the Reynolds number, which can be calculated by

$$\mathrm{Re} = \frac{\rho \cdot l \cdot u}{\mu} \qquad \text{(Eq. 1)}$$

Here, l represents the characteristic length of the fluid flow, u the characteristic velocity, and ρ and μ the material properties of the fluid. For stirred bioreactors, the length and the velocity is replaced by a geometrical (stirrer diameter d) and a bioreactor operating (stirring speed n) parameter for the bioreactor, which can be written as $l \cdot u = n \cdot d^2$. As a result, the stirrer Reynolds number is given by

$$\mathrm{Re} = \frac{\rho \cdot n \cdot d^2}{\mu} = \frac{n \cdot d^2}{\nu} \qquad \text{(Eq. 2)}$$

It can easily be seen that the material properties, namely the density and the dynamic viscosity (both can be combined to the kinematic viscosity $\nu = \mu / \rho$) are factors that influence the Reynolds number.

During the cultivation of plant cells, the density of the fluid can be considered to be constant. However, the viscosity of the fluid is changed considerably due to growth and the secretion of polysaccharides [82–84]. Therefore, from an engineering point of view, the most important material property of plant suspension cell cultures, unlike other processes, is fluid viscosity. Variations are as a result of polysaccharides, which are secreted into the medium and change the rheological behaviour and, often more

importantly, change the aggregation tendency, shape and number of large cells. Generally, plant suspension cell fluids are considered to be suspensions which are a more or less water-like fluid loaded with a varying number of heterodisperse particles.

Suspended particle fluids often exhibit non-Newtonian flow characteristics with shear-thinning behaviour (some well-known examples are blood or sand in water), this means that the viscosity is dependent on the shear rate $\dot{\gamma}$. Typical flow and viscosity curves are presented in Fig. 4. The classical power-law approach (often referred to as the Ostwald-de Waele relationship) is often used to describe rheological behaviour, and can be written as

$$\mu = k_{\gamma} \cdot \dot{\gamma}^{(a-1)} \qquad \text{(Eq. 3)}$$

Here, k_{γ} represents the flow consistency index and the factor a is the flow behaviour index, with a = 1 for Newtonian fluids, a < 1 for pseudoplastic or shear-thinning fluids and a > 1 for dilatant or shear-thickening fluids. For plant cell suspensions, the flow behaviour index is often in a range between 0.5 and 1, meaning the fluids exhibit a slight shear-thinning behaviour [38].

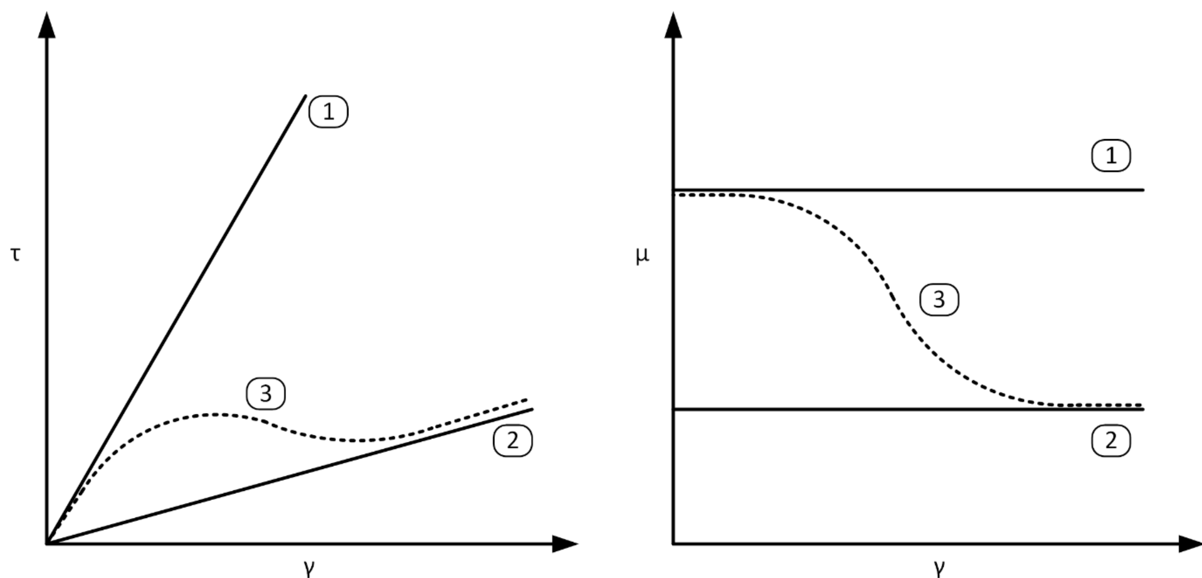

Fig. 4: Typical flow curve (left) and viscosity curve (right) of Newtonian fluids (1 and 2) and non-Newtonian fluids with pseudo-plastic flow behaviour (3), adapted from [85]

As a result of the shear rate dependency of viscosity, it is obvious that the Reynolds number and the subsequently derived engineering parameters might not be

estimated correctly. Therefore, it is necessary to adapt calculations of engineering parameters in order to account for variations in viscosity.

The most commonly used approach dates back to 1957 and was proposed by Metzner and Otto [86]. It describes the effective shear rate $\dot{\gamma}_{MO}$ in relation to the stirring speed n and an empirical determined factor k_{MO} as follows

$$\dot{\gamma}_{MO} = k_{MO} \cdot n \qquad \text{(Eq. 4)}$$

Here, the so-called Metzner-Otto constant k_{MO} is only dependent on the type of the stirrer and must be determined experimentally. Typical values found in the literature are as follows: approximately 10 for a marine impeller, 12 for a Rushton turbine, 11.5 for a pitched-blade impeller, 25 for an anchor impeller and 30 for a helical ribbon impeller [85, 87].

However, the linear relationship of the effective shear rate to the stirring speed with a correlation factor based only on the geometry of the stirrer is obviously highly simplified and, in the strict sense of its original derivation, only valid for laminar flows [86]. Furthermore, many researchers have proposed a dependency between the Metzner-Otto constant and the flow behaviour index [88]. This is particularly important for highly shear-thinning fluids, and thus the Metzner-Otto approach has been used extensively for plant cell suspensions with their minor shear-thinning behaviour [38, 89].

Besides the shear rate, cell density also heavily influences apparent viscosity. Depending on the correlation of the apparent viscosity to different cell mass measurements (cell dry weight, cell fresh weight, packed cell volume), masking effects may occur due to variations in individual cell sizes and water contents (e.g. in the vacuole) over the course of the growth cycle.

After calculating the Reynolds number based on the previous description, the power number (often referred to as the Newton number) can be calculated as follows:

$$Po = \frac{P}{\rho \cdot n^3 \cdot d^5} \qquad \text{(Eq. 5)}$$

The power number is known for a wide variety of stirrers, and therefore it can be used to calculate the power input P for the bioreactor, which can be helpful in estimating mixing and oxygen mass transfer to the bioreactor. Furthermore, the power input P or, even better, its volume-normalized pendant, the specific power

input P/V, is related to the shear stress applied to the liquid, which is often an important parameter for shear sensitive plant cells.

Mixing ensures a sufficient supply of nutrients and prevents the accumulation of toxic metabolites during fluid homogenisation and particle suspension. In order to determine the quality of mixing, the mixing time is often used [90, 91]. For small scale bioreactors, it is generally easy to ensure good mixing and thus low mixing times. However, this task frequently becomes one of the constraints during scale-up of bioreactors, which leads to concentration gradients and poor mass transfer. Improved mixing can be achieved by increasing the agitation rate, however this may not be the appropriate method for shear-sensitive plant suspension cells. Alternatively changes can be also be made to the stirrers or the overall mixing principle of the bioreactor. For example, mixing can become the limiting factor in larger airlift bioreactors when reaching a cell density of more than 20 g L^{-1} cell dry weight. Increasing the aeration rate is the only operating response that can increase mixing [4]. However, studies have shown that overventilation can reduce cell growth and product formation due to the CO_2 and other growth related gases like ethylene being stripped [92]. This obviously emphasises the importance of considering the final bioreactor scale when designing processes and the careful selection of a suitable bioreactor systems from the very beginning.

The supply of oxygen is crucial for all heterotrophic cells. However, the oxygen demand of plant cells is, in general, relatively low [93]. Nevertheless, the high cell densities which can be achieved with plant suspension cells, the high viscosity of the culture broth, and strong foaming and cell floating tendencies can be potential issues. The characterization of plant cell cultures and the characterization of the bioreactors to be used is an essential part of process design. How to measure the specific oxygen transfer coefficient has recently been described [90]. However, the measurement is often made using water, and as a result transferring the coefficient to plant cell culture processes is complicated. In such cases, the use of a model liquid like carboxymethyl cellulose water solution is recommended, which can be adjusted to certain growth stages of plant suspension cells by altering the concentration [56].

The sensitivity of plant suspension cells to shear forces is considered relatively high due to the large size of the cells compared to microorganisms. Furthermore, large vacuoles, which occur during the late growth phase in particular, may even increase sensitivity [94]. In general, shear stress can be reduced by reducing the power input. However, reducing power input can lead to inadequate mixing and can also reduce oxygen and heat transfer rates in high viscosity plant cell culture broths. Another

alternative to reducing shear stress to plant cells is to employ special types of bioreactors that are characterized by very homogeneous power inputs. This eliminates high shear regions, which usually occur when mixing is performed by a stirrer or similar device.

4.2 Tissue culture specialities

Plant tissue cultures can be used for various applications, e.g. the production of pharmaceutical active ingredients like secondary metabolites, the vegetative reproduction of highly productive clones and the growth of genetically modified plants [44]. Several types of biotechnologically applicable plant tissues exist and their usage depends heavily on the objective of the cultivation: Adventitious and hairy roots are the predominant forms used in research and for the production of secondary metabolites; embryogenic and shoot cultures are primarily used for plant micropropagation. Besides their different uses, the appearance of plant tissues differs greatly and leads to particular cultivation requirements. The characteristics, advantages, and challenges of plant tissues, with a particular focus on root cultures, will be discussed.

Differentiated cells are characterized by their high genetic stability and their ability to grow in hormone-free media. Furthermore, constant secondary metabolite production and high biomass productivity can be observed for hairy roots [95, 96]. The reason for the biotechnological use of tissues for the producing secondary metabolites is obvious because they are typically produced in differentiated cells [97, 98].

Due to their dissimilar structure, the requirements for plant tissue cultures depend greatly on their shape. Although high shear forces do not inevitably reduce the growth rate, they may destroy agglomerations and hence reduce growth potential. Root cultures tend to form large, connected networks which react sensitively to mechanical forces. Furthermore, some root species favour anchoring structures for faster growth and thus reduce the range of potential bioreactors.

In contrast to plant cell suspension cultivations, the viscosity increase in tissue cultures is considerably lower and thus of minor importance in terms of process design. However, major concentration gradients can appear in larger cell tissues (e.g. in dense root networks) and may lead to nutrition deficits and reduced oxygen concentrations in the tissue centre [99]. Nevertheless, higher energy input (e.g. due to an increased stirring rate) can be problematic, since it may disrupt cell agglomerates

or tissues and thus lead to reduced growth or increased cell death. Therefore, the selection of a suitable reactor system is of major importance.

5 Concluding remarks

Selecting an optimal bioreactor system for a particular plant cell or tissue culture is a complex task. There are many different bioreactor systems commercially available. Small scale bioreactors, in particular disposable bag bioreactors, are more or less ready to use and can be used for both cell and tissue cultures. Because this choice is influenced by the culture type (suspension or tissue culture), the growth type (e.g. the need for light) and the targeted production scale, there are only a limited number of bioreactor types remaining that represent appropriate choices. Nevertheless, large production scale bioreactors for plant cell suspension cultures are mainly stirred or pneumatically mixed and are usually tailor-made to the specific process.

Mixing, aeration and—related to both—shear forces can be considered to play a crucial role in many cultivation processes. Thus, these parameters need to be well equilibrated in order to gain an optimal result for cell growth and product formation. An ideal bioreactor provides good and homogenous mixing and thus sufficient gas exchange and nutrient supplementation, while keeping shear forces low. Therefore, it is not surprising that it is almost impossible to provide a general. In fact, every single process needs to be optimized and scaled up individually to the desired production size.

The "bioreactor chooser" can be useful tool during the first steps of the selection of an appropriate bioreactor system. More detailed information for a deliberated selection can be found in section 3.

Various challenges during scaling up the process due the particular nature of plant cell or tissue cultures have been described. It is particularly important that the drawbacks of increasing viscosity for suspensions cells and nutrient shortage in larger root networks be considered. The pitfalls associated with (large-scale) plant cell cultivations may differ from mammalian or microbial cultivations, but they can be avoided as long as the reactor selection is carefully thought through.

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