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REVIEW

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Foam fractionation methods in aerobic fermentation processes

Amira Oraby[1,2] | Isabell Weickardt[1] | Susanne Zibek[1,2]

[1]Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Stuttgart, Germany

[2]Institute of Interfacial Process Engineering and Plasma Technology IGVP, University of Stuttgart, Stuttgart, Germany

Correspondence

Susanne Zibek, Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Nobelstr. 12, 70569 Stuttgart, Germany.
Email: Susanne.Zibek@igb.fraunhofer.de

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Abstract

Inherently occurring foam formation during aerobic fermentation of surface-active compounds can be exploited by fractionating the foam. This also serves as the first downstream processing step for product concentration and is used for in situ product recovery. Compared to other foam prevention methods, it does not interfere with fermentation parameters or alter broth composition. Nevertheless, parameters affecting the foaming behavior are complex. Therefore, the specific foam fractionation designs need to be engineered for each fermentation individually. This still hinders a widespread industrial application. However, few available commercial approaches demonstrate the applicability of foam columns on an industrial scale. This systematic literature review highlights relevant design aspects and process demands that need to be considered for an application to fermentations and proposes a classification of foam fractionation designs and methods. It further analyses substance-specific characteristics associated with foam fractionation. Finally, solutions for current challenges are presented, and future perspectives are discussed.

KEYWORDS

fermentation, foam fractionation, foaming, integrated, ISPR

1 | INTRODUCTION

Aerobic fermentations are known to be prone to excessive foaming due to surface-active substances in the culture broth. Foam is a complex structure of gas entrapped into liquid films at a volume fraction of 0.50–0.97 (Junker, 2007; Walstra, 1989). Surface-active molecules present in the liquid adsorb at the formed gas–liquid interfaces, resulting in an amphiphile-enriched layer that stabilizes the foam (Junker, 2007; Walstra, 1989). Due to the present density gradient, foam bubbles ascend to the surface of the liquid, thus separating these amphiphilic molecules from fermentation broths. This phenomenon can be used or actively induced to remove these molecules present in comparably small concentrations from large amounts of liquid by separating the foam (Grieves, 1975; Uraizee & Narsimhan, 1990). Foam fractionation, as described, belongs to the adsorptive bubble separation methods (Lemlich, 1968). It can be defined as the selective separation of one or more amphiphilic solutes that are adsorbed to the gas–liquid interface of the foam. In contrast, in foam flotation, nonsoluble and hydrophobic particles attach to the gas bubble and are separated by the foam (Lemlich, 1968; Stevenson & Li, 2014).

If not exploited, prevented, or disrupted, foam can cause severe damage to fermenters. Overfoaming leads to unstable fermentation processes due to the resulting blockage of exhaust filters and alteration of media compositions that may cause changes in microbial metabolism induced by nutrient limitations. Drawbacks can further

be a necessary decrease in working reactor volume to ensure enough headspace for the incurred foam or energy loss due to lower mixing quality or inhomogeneity (Lemieux et al., 2019; Vardar-Sukan, 1998).

Thus, several foam prevention methods are commonly applied in fermentative processes. Adjustment of reactor aeration and agitation strategies directly affects foam formation and can be directed at reducing it. However, this would simultaneously impact oxygen transfer to the media and thus influence the performance of the whole fermentation process. Mechanical or physical methods usually apply shear stress to disrupt foam, for instance, by using integrated foam breakers in the reactor. While mechanical foam breakers do not affect culture composition, they have high operating costs and may cause shear stress to the microorganisms (Vardar-Sukan, 1998). Applying back pressure to the reactor can also be used to control foam if the used microorganisms can withstand high pressure (Junker, 2007). Thermal and electrical treatments or the use of ultrasound are also efficient but not widespread due to microorganism sensitivity to such parameter variations (Vardar-Sukan, 1998). Chemical methods are more common and economical for foam mitigation: Here, the application of various antifoam agents or defoamers usually proves to be efficient in foam mitigation, but alters the composition of the culture broth and may complicate downstream processing (Junker, 2007).

While the different foam prevention or disruption methods each have their advantages and drawbacks, using the existing foam for product separation may often be the more suitable and sustainable alternative for fermentation. Foam fractionation goes back as early as 1900, with the first registered patent dating to 1920 and the first known application for biomolecule separation during a fermentation process published in 1981 (Cooper et al., 1981; Lemlich, 1968; Linke et al., 2005). Although foaming and foam fractionation are widely studied phenomena with various implemented techniques, more research is still required for a better understanding of occurring mechanisms and suitable designs. Existing literature mostly either only describes the implementation of foam fractionation to separate a particular type of fermentation product (C.-Y. Chen et al., 2006; Heyd et al., 2011; Winterburn et al., 2011a) or focuses on the influence of various fermentation conditions on foam formation, or applied methods for foam prevention (Junker, 2007; Vardar-Sukan, 1998). Further, the advantages of foam fractionation have been compared to other in situ product recovery (ISPR) methods (van Hecke et al., 2014). However, due to a large number of different sources of available information on foam fractionation strategies, grasping all relevant aspects and comparing design strategies is challenging.

This review provides an overview of current advances in foam fractionation, showing different fermentatively produced compounds (Section 3), for which foam fractionation is known to have been applied. It further presents a coherent classification of foam fractionation designs and methods and highlights relevant design aspects (Section 4), including foam disruption methods applied in foam traps (Section 5). The scope of analyzed publications is limited to fermentative processes implementing foam fractionation, with a focus on integrated foam fractionation methods during fermentation. While process advantages associated with foam fractionation are presented (Section 6), current challenges that need to be overcome for the establishment of foam fractionation in fermentative processes are discussed (Section 7). Process scalability and economics are crucial for a widespread industrial application and thus are discussed in Section 8. It further presents a guideline that can aid in designing foam fractionation implementations for prospective fermentations.

2 | METHODOLOGY

The search strings used to generate the literature database for this systematic literature review are displayed in Figure 1. Two scientific databases were searched in January 2021, yielding a total of 541 hits.

From those, only primary sources, which included microbial whole-cell biotechnological cultivations using foam fractionation, were considered. So enzymatic bioconversions and foam fractionations of artificial broths were excluded, resulting in 72 hits fulfilling the previously specified requirements. Eight more were found by screening their references for publications meeting the mentioned criteria (snowballing).

Out of the 80 publications fulfilling all criteria, 61 comprised cultivations in stirred tank bioreactors, 1 in an airlift bioreactor, and 18 in shake flasks.

The processes conducted in shake flasks were only considered for qualitative analysis of foaming-related aspects. For quantitative analysis and design-related aspects, like the implementation of foam fractionation in the fermentation process, including technical details like foam column dimension and foam rupture, only fermentations conducted in stirred tank bioreactors were considered. If multiple designs were described in a single publication, only the design with the best performance was included (e.g., Atwa et al., 2013). Furthermore, fermentation-related parameters like product type, reactor volume, and feeding approach were analyzed. Based on that, process advantages and promising areas for integrating foam fractionation in bioprocesses were identified. An overview of all analyzed 80 publications and their classification are given in Table S1.

The first publication of a fermentation process with foam fractionation was published in 1981 (Cooper et al., 1981). Until 2005, only 7 out of the 80 relevant publications were published; afterwards, the research interest in this area significantly increased. The maximum number of papers per year was reached in 2020, showing an abiding interest in the topic. While papers up to 2005 mainly considered lipopeptide fermentations and applied foam traps, the variety of products and fermenter setups increased afterwards.

3 | PRODUCTS RECOVERED BY FOAM FRACTIONATION

Fermentation products highly affect the foaming behavior of the culture broth and thus the suitability of the application of foam fractionation. Therefore, it is inevitable to determine if the desired product qualifies for foam fractionation when considering its application. Foam fractionation applications for product recovery

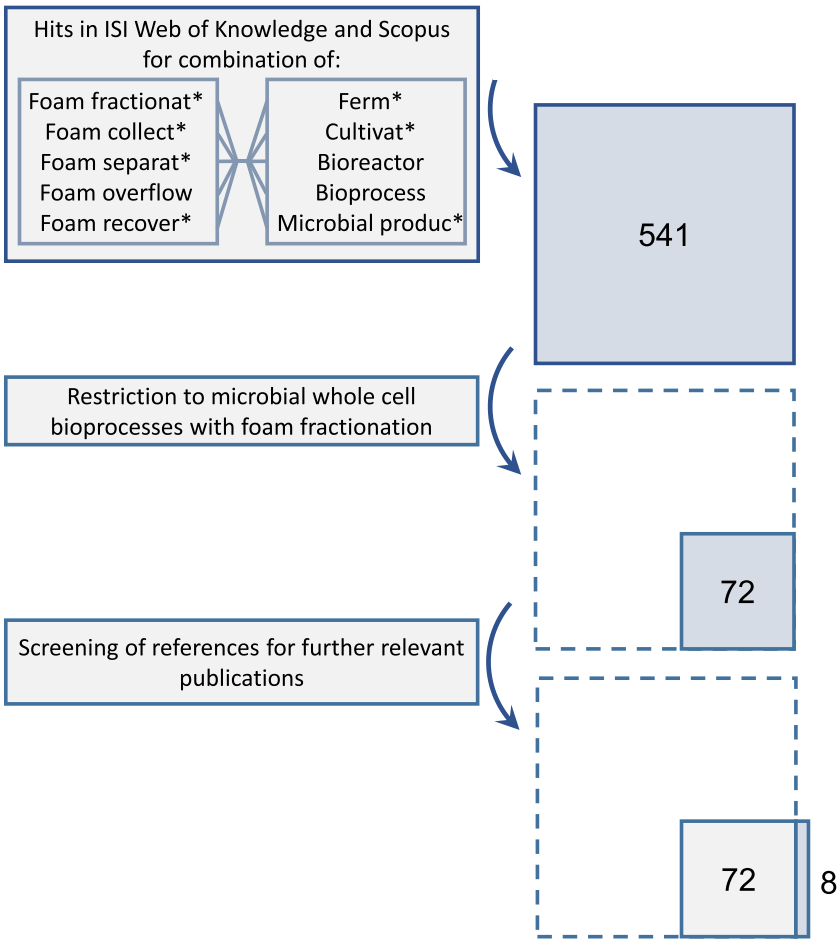

FIGURE 1 Database generation and search methodology.

during fermentation are described mostly for processes in which biosurfactants, proteins, including enzymes, or peptides are produced. In bioreactor processes, biosurfactants are the dominant group with more than 75% of the analyzed publications (Figure 2), mainly lipopeptides like surfactin (Alonso & Martin, 2016) and glycolipids like rhamnolipids (Jia et al., 2020).

The large interest in foam fractionation application for biosurfactant fermentations may be explained by the naturally occurring large amounts of foam during their fermentation. Here foam fractionation serves predominantly as means to exploit the produced foam and thus prevent common challenges associated with excessive foaming. At the same time, it facilitates the proceeding purification. Biosurfactants are usually produced in relatively small concentrations; thus, foam fractionation serves as a first concentration step. Foam fractionation may also result in increased product yields by avoiding productivity loss caused by the usage of antifoam agents (Atwa et al., 2013). In addition, the resulting ISPR further prevents potential feedback inhibition, which may occur in batch cultures (Zheng et al., 2015).

Protein and enzyme fermentation, the second-largest product group in the analyzed literature, may show less excessive foaming compared to biosurfactants. Therefore, additional foam induction or foam propagation proceeding or during the fermentation process is usually necessary for foam fractionation applications. By sparging nitrogen to the supernatant of a *Pleurotus sapidus* culture and fractionating the foam, Linke et al. (2005) obtained a maximum recovery of hydrolytic activity of 95% for lipase. The need for additional foam induction is reflected in the applied technical implementations for protein separation via foam fractionation, where sequential and external foam columns are mostly used (Khalesi et al., 2013; Winterburn, 2011). However, a problem typically encountered when foaming enzymes is a loss of catalytic activity (Section 7.2). Because of the smaller and simpler structure and an absence of catalytic function, biosurfactants are usually not functionally affected by foaming, in contrast to proteins. This may be a further reason for the low number of protein fermentations using foam fractionation, compared to biosurfactants (Figure 3) (Rangarajan & Sen, 2013).

For the third largest product group, namely, peptide fermentations, foam fractionation is especially suited to prevent feedback inhibition, which is a relevant challenge in nisin fermentations, for instance. Applying ISPR via foam fractionation resulted in an increase in nisin productivity by up to 36%, compared to the fermentation without ISPR (Zheng et al., 2015).

In general, foam fractionation is especially suited for the recovery of substances present in low concentrations in the culture broth and which exhibit high structural stability. A high foaming ability of the target substance further makes additional aeration unnecessary. Applying foam fractionation can also serve as a means to prevent potential feedback inhibition via ISPR.

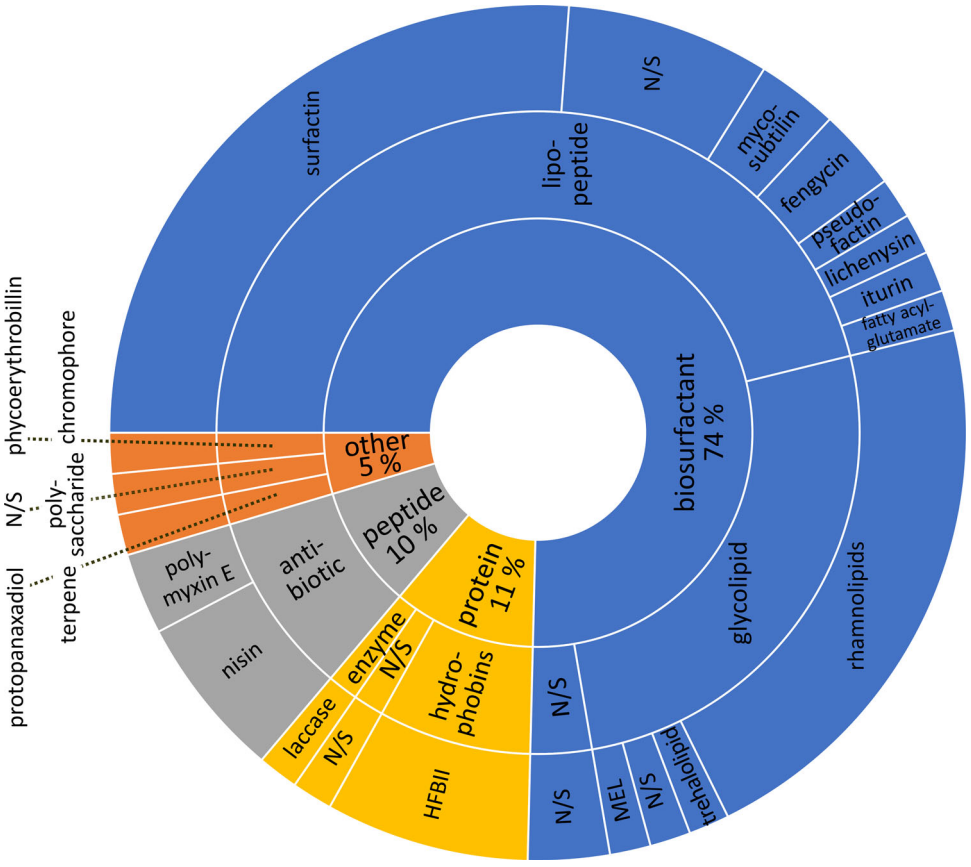

FIGURE 2 Product distribution among bioreactor processes applying foam fractionation methods coupled to stirred tank reactors, categorized according to the number of analyzed publications (%). Products that were mentioned in less than 5% of the analyzed publications are classified under the category "other." N/S, not specified in the respective publication.

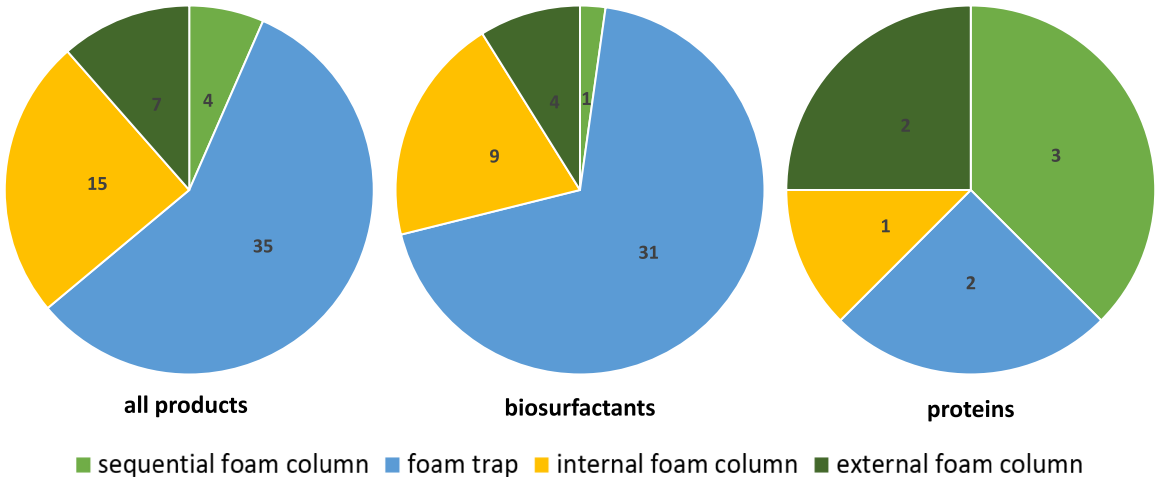

FIGURE 3 Distribution of the shares of different foam fractionation implementations among product groups.

4 | TECHNICAL IMPLEMENTATION OF FOAM FRACTIONATION IN FERMENTATIVE PROCESSES

Numerous examples for foam fractionation designs are available; however, there is no universal construction standard that can be applied to all fermentations. Current designs are usually specific for a described application, but constructive similarities and patterns can be found and are summarized in this section to provide an overview of possible implementations.

In general, foam fractionation can be applied during or after fermentation. Postfermentation applications have the advantage that they are independent of various parameters applied during fermentation. Here, the culture broth is separated from the fermenter and

sparged with a gas to create foam, which is then collected to obtain the desired product. This variant was applied, for instance, by Khalesi et al. (2016) and Khondee et al. (2015) and classified by the authors as "nonintegrated foam fractionation" (Concept 1, Figure 4a). This method is carried out in a sequential foam column and is usually used as a means for product separation and recovery in downstream processing (e.g., Linke & Berger, 2011).

When applied during fermentation, "integrated foam fractionation" (Concepts 2–4, Figure 4a) serves not only as a tool for ISPR but also for foam exploitation. By directing the already present foam from the fermenter to the foam fractionation unit, foam overflow, and herewith associated problems can be avoided. Integrated foam fractionation is applied more often for fermentations than the nonintegrated setup. Out of the examined publications, the majority describe methods for integrated foam fractionation. Their design concepts are discussed in the following section.

All integrated foam fractionation designs include a foam collecting vessel, the so-called foam trap, which is connected to the fermenter and described in more detail in Section 4.1. Common designs differ either in their connection to the fermenter (direct connection (Concept 2) or foam column (Concepts 3 and 4; Figure 4) or in their operation mode without (a) or with (b) recirculation of the collapsed foam, the foamate. A proposed classification based on these differentiations is illustrated in Figure 4. Described foam columns in literature (Section 4.2) were classified under Concepts 3 or 4 in the present work only if the direction of the foam flow is opposite to gravity. Other packed bed columns used for product adsorption with a foam flow in the direction of gravity were categorized as foam traps (2a or 2b) (e.g., Anic et al., 2017).

4.1 | Foam trap

In the basic design overflowing foam is directed from the fermenter to a foam trap, usually through tubes (Concept 2, Figure 4) (Bages-Estopa et al., 2018; Barros et al., 2008; Biniarz et al., 2020). Due to their comparably simple implementation, foam traps are the earliest foam fractionation method applied to fermentations (Cooper et al., 1981) and remain the most described ones in the literature, with more than 50% of the respective publications up to 2020.

The basic concept remains the same, but different constructions were used for the different fermentations. In most described constructions, the foam is collected through the air exhaust line. In some cases, this is done through the exhaust gas cooler (Beuker et al., 2016; Chenikher et al., 2010), whereas in others, the exhaust gas cooler is completely dismantled from the fermenter setup (Barros et al., 2008; Biniarz et al., 2020). An impact on liquid loss via vapor was described nowhere. In all cases, the foam flows autonomously out of the fermenter due to pressure gradients caused by aeration without further energy input. Only Kottmeier et al. (2012) reported an additional pump for conveying the foam from the reactor headspace to the foam trap. The foam trap usually consists of a simple vessel, which can be put on a scale to measure the separated foam amount (Biniarz et al., 2020), or of interchangeable vessels, which further enable the analysis of the separated foam over specified periods (Beuker et al., 2016).

The size of the foam trap is usually limited by the time the foam needs to collapse (C.-Y. Chen et al., 2006). For less stable foam, comparably smaller vessels are sufficient for collecting the foam. The higher the foam stability, the longer becomes the residence time of

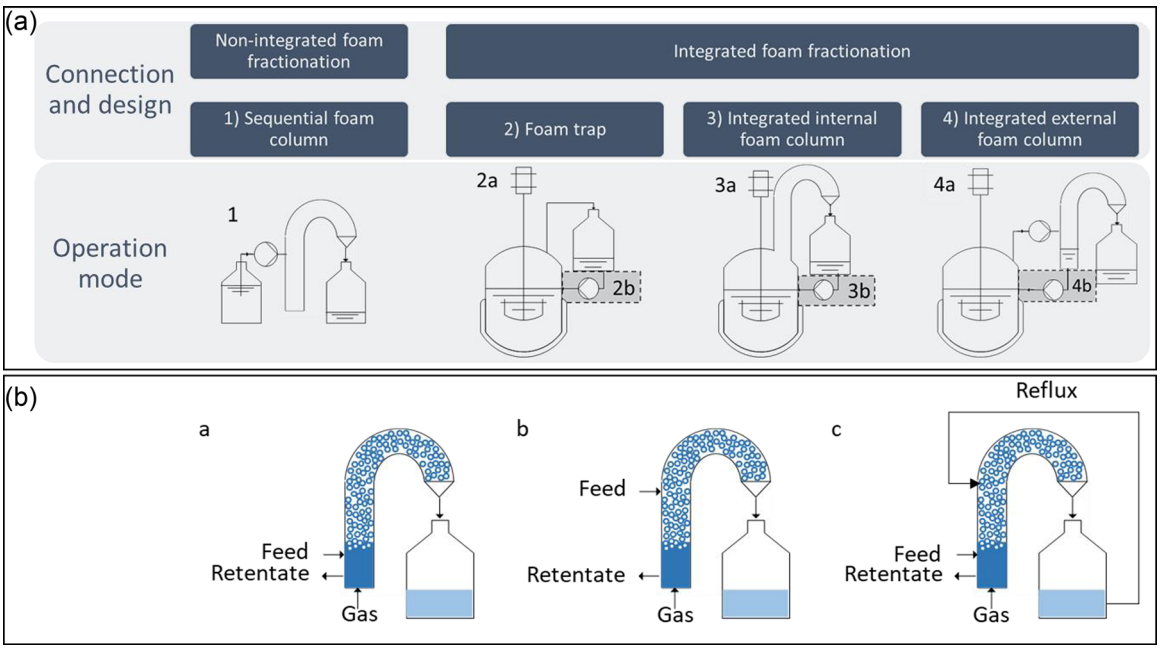

FIGURE 4 (a) Classification of different technical designs implemented for foam fractionation units for fermentations. Of each design, operation mode (a) is without and operation mode (b) with recirculation. (b) Foam column with (a) simple mode, (b) stripping mode, and (c) enrichment mode, adapted from (Lemlich, 1968). A combination of versions (b) and (c) is also possible for the foam columns.

TABLE 1 Effect of different design and operational parameter variations during fermentation, coupled with foam fractionation, on product enrichment E_P and recovery R_P

Operation and design parameter	Variation	Effect	E_P	R_P
Gas flow rate	↑	~ to the number of gas bubbles ~ to the decrease of residence time of foam in foam columns or the fermenter headspace □ affects foam ripening and drainage (Zheng et al., 2020)	↓	↑
Agitation rate	↑	~ to gas bubble dispersion (smaller gas bubbles) and distribution in the culture broth ~ to higher specific surface area and longer residence time of the bubbles □ more interstitial liquid resulting in a wetter foam (Burghoff, 2012)	↓	↑
Sparger pore diameter	↓	~ to the size of gas bubbles that are introduced to the liquid □ higher specific surface area (Burghoff, 2012) □ more interstitial liquid resulting in a wetter foam (Burghoff, 2012)	↓	↑
Recirculation		□ Media components, liquid and in some cases biomass, are directed back to the fermenter □ the target product remains in the foam collecting vessel	↑	-
Foam column H	↑	~ to the foam residence time □ drainage effects are increased and the foam becomes dryer □ combined with high H/D ratios, the wall effect results in more dryer foam (D. Zhang et al., 2015)	↑	↓
Enrichment mode		□ surface-active molecules can adsorb on the bubble surface and enrich the interstitial liquid, while liquid drains back to the bottom of the column (Lemlich, 1968) □ effects occurring due to recirculation are intensified	↑	-
Stripping mode		□ the interstitial liquid has a higher enrichment, compared to the pool concentration (Lemlich, 1968) □ the bottom is purer (Lemlich, 1968)	-	↑

Note: ~, proportional to; ↑, increase; ↓, decrease; -, no known effect.
Abbreviations: D, diameter; H, height.

the foam, the larger the foam traps need to be designed. This can be a limiting factor for a continuous foam separation. A solution to maintain a continuous operation without using large foam traps is the active disruption of the foam in the foam traps, which is discussed in Section 5.

This basic concept of a simple foam trap suffices for overcoming the foaming problem and enables ISPR. However, culture media and biomass contained in the foam fraction lead to productivity loss. Recirculation of the foamate and thus the recycling of the separated biomass and culture media, while retaining the separated product in the foam trap, provides a solution, therefore. This can be achieved by pumping back the foamate to the reactor (Concept 2b, Figure 4) and is confirmed to increase overall process productivity (Atwa et al., 2013; Gong et al., 2009). Applying an additional pressure gradient between the foam trap and the fermenter may also be used as a method for foamate recirculation (Gong et al., 2009). In both cases, the target product remains in the foam trap through adhesion to the vessel's walls.

The selective retention of the target product can further be enhanced by using adsorptive packings in or before the foam trap. Anic et al. (2018), for instance, directed the foam from the top of the bioreactor, through a packed adsorption unit, and then to the foam trap, whereby exhaust air was emitted. While the product rhamnolipids thereby accumulated continuously in the adsorption unit, the residual foamate containing biomass and culture media was pumped back to the fermenter.

Another method for decreasing biomass retainment in the foam trap/foamate is via integrating the whole foam fractionation system into the succeeding DSP system, where biomass gets separated from the foamate and can then be reintroduced to the fermenter (Küpper et al., 2013). Biomass loss and recycling are discussed in detail in Section 7.1.

Foam traps, as shown, provide the simplest means for foam exploitation and ISPR via foam fractionation. Recirculation of the foamate additionally decreases biomass and culture media loss. Nonetheless, the foam composition can barely be manipulated using this design, and the foam is directed to the foam trap without any alteration to its components and liquid content. Using foam columns actively influences these factors by foam ripening and drying effects.

4.2 | Foam columns

The second most common implementation of foam fractionation during fermentation is the usage of foam columns, dating back to 2001 when an integrated foam column was first applied for surfactin

recovery (Davis et al., 2001). Foam columns can either be integrated directly into the fermenter (Concept 3, Figure 4, integrated internal foam columns) or outside the fermenter (Concept 4, Figure 4, integrated external foam columns). Integrated internal foam columns are used more widely and are described by approx. 25% of the analyzed publications describe implementations of foam fractionation in fermentations up to 2020.

In foam columns, the newly formed foam bubbles in the reactor cause the foam to flow upwards within the column, while entrapped liquid flows downwards due to drainage effects (Burghoff, 2012). This eventually leads to dryer foam at the top of the column with larger bubble sizes and thus smaller specific surface area. While media components and biomass largely remain in the downwards flowing liquid, the surface-active molecules accumulate in the gas–liquid interface. This results in an increased enrichment of the foam fraction. These effects partly happen in the headspace of the fermenter and are therefore dependent on the size of the headspace, regardless of the applied foam fractionation method. They can, however, be intensified when using a foam column. A detailed overview of theoretical considerations regarding the design of foam columns is given in Burghoff (2012).

Different design considerations of foam columns ultimately aim at increasing the enrichment of the target molecule and/or its recovery, usually depending on the proceeding purification method. However, a high enrichment is generally accompanied by a decreased recovery and vice versa (Burghoff, 2012; Winterburn et al., 2011b). Enrichment describes the ratio between the concentration of the target product in the foamate and its concentration in the culture broth. The recovery rate states the percentage of product recovered from the foamate in relation to the total amount (Burghoff, 2012).

In foam columns, the most relevant constructive parameter affecting both enrichment and recovery is the H/D ratio. Values vary from 1.4 up to 33.3 in the analyzed internal and external integrated foam column designs in literature (Blesken et al., 2020; Heyd et al., 2011). However, no correlation of the chosen H/D ratio to neither product group, technical implementation, nor fermenter size was observed. Only a few publications considered and compared the effect of different column heights or H/D ratios on enrichment and recovery (Heyd et al., 2011; Khondee et al., 2015; Sarachat et al., 2010).

In general, higher column heights H result in a longer residence time of the foam in the column. This causes an increase in drainage effects and thus results in a dryer foam and higher enrichment. A higher column height further results in a larger wall contact area between the rising foam and the column wall. Here, the drainage velocity of the entrapped liquid is higher between gas bubbles and the column's wall compared to the foam bulk, leading to an even dryer foam due to the wall effect (Lu et al., 2013; D. Zhang et al., 2015). However, increasing the column diameter D while maintaining the same column height would cause a decrease in the foam rising velocity, thus resulting in a higher residence time. This further contributes to higher enrichment. The column diameter also impacts bubble size (Sarkar et al., 1987). Consequently, both column height H and diameter D, as well as their ratio, affect enrichment and recovery, while the previously mentioned effects interact diversely in different systems. Therefore, with the current knowledge, the optimal column dimensions need to be determined for each foam fractionation system individually.

Especially with protein fermentation, further substance-related effects need to be considered when designing a foam column and adjusting the working volume level in the fermenter. For example, oxidative deactivation and shear forces at interfaces may cause conformational changes in proteins' tertiary structures, which increase at higher foam column heights (Norde & Giacomelli, 1999; D. Zhang et al., 2015).

Due to the occurring effects of product enrichment along the foam column and biomass removal, its concentration in the foam is usually lower compared to foam directly separated in foam traps. Nonetheless, biomass immobilization approaches can be used to eliminate or reduce biomass present in the foam fraction, as applied by Gao et al. (2018) for nisin fermentation with *Lactococcus lactis* immobilized on a carrier of loofa sponge and κ-carrageenan.

Regarding the recirculation and feed addition to the column, a differentiation is made between the simple, the stripping, the enrichment, and the combined mode (Figure 4b), based on the height level of feed addition within the foam column (Lemlich, 1968). In simple mode, the feed is added at the bottom of the column to the liquid pool, whereas in stripping mode, the feed is added above the liquid pool or to the upper part of the column, directly through the rising foam. In enrichment mode, a fraction of the collapsed foamate is additionally recirculated back to the top of the column to increase product enrichment for highly diluted fermentation broths (Burghoff, 2012; Lemlich, 1968). In the combined approach, the feed and recirculated foamate are added to the rising foam within the column, aiming at increasing both enrichment and recovery rate (Bagés-Estopà, 2017; Lemlich, 1968).

Further, various designs for improved drainage in foam columns have been discussed in the literature. Drainage is mainly enhanced by increasing the bubble size or liquid flux (Stevenson & Li, 2014). This can be reached by installations inside the fractionation column (Liu et al., 2013) or by varying the cross-sectional area. For example, an hourglass shape or the installation of a spherical channel on top of the foam column efficiently increased foam drainage (Banerjee, 1993; Gao et al., 2018; Gerken et al., 2005; Jia et al., 2020; Linke et al., 2005). Here, the successive contraction and expansion of the foam in the column resulted in a decrease in the liquid holdup, compared to a regular column with similar dimensions (Gao et al., 2018).

When applying integrated external foam columns, the choice of a sparger unit becomes an additional crucial implementation parameter. An optimum pore size diameter for sufficient foam generation and enrichment while maintaining a controllable foam amount needs to be identified. The pore size directly affects the gas bubble size, which in turn affects the rising velocity of the bubbles from the bulk liquid solution and the foam bubble size at the liquid surface (Chisti, 1992). Occurring effects are discussed in more detail in the next section.

4.3 | Operation parameters of foam fractionation units

Besides the various design aspects of the foam fractionation unit, certain operation parameters during the fermentation and design aspects of the fermenter affect the foaming behavior and thus foam fractionation (Table 1). Aeration is the cause for foam generation, with the bubble size being directly proportional to foam stability when surface-active agents are present. Thus, higher aeration rates generate larger volumes of stable foam if the air bubbles are introduced to the liquid in small sizes using a porous sparging unit, or the bubbles are dispersed via the stirrer. Hence, agitation also directly influences foam formation. However, higher aeration and agitation rates resulting in increased foam generation are not beneficial for foam fractionation in general. The substances to be separated need a certain residence time (gas holdup in the fermentation broth) to get enriched on the bubble surface and thus in the foam fraction. So a high recovery rate, bound to the high amount of generated foam, is usually not accompanied by a high enrichment. Further, extensive foam generation results in a rapid loss of culture broth, especially in foam fractionation systems without recirculation (Davis et al., 2001). At the same time, oxygen supply to the fermenter is a crucial parameter for biomass growth and metabolite synthesis. Therefore, an optimum between sufficient oxygen supply for the microorganism, adequate bubble sizes for the generation of stable foam, and a gas flow rate for sufficient enrichment need to be identified for each fermentation process individually (Cui et al., 2014; Davis et al., 2001; Santos da Silva et al., 2015; Zheng et al., 2020). Furthermore, variation in broth viscosity affects bubble hydrodynamics (Besagni et al., 2017).

Additionally, the media composition has a direct effect on foaming behavior as well (Stiefelmaier et al., 2018). The applied pH value may affect both growth and metabolite synthesis, as well as the solubility and stability of the synthesized target metabolite (Liu et al., 2010; D. Zhang et al., 2015). Both are crucial parameters that highly affect foaming. Therefore, while implementing foam fractionation, a possible effect of these factors needs to be examined when overall process optimization is considered.

Foam formation rates usually fluctuate throughout the fermentation, depending on the composition of the culture broth (Andrade et al., 2017; C.-Y. Chen et al., 2006). Excessive foaming is favored during phases in which the culture broth is enriched with the target product to be separated but undesired after feeding, where foam separation may cause loss of nutrients or biomass. One simple approach to control foaming is by adjusting the height of broth liquid relative to the impeller to control the impact of the impeller on mixing versus foam formation. This can be achieved, for instance, by adjusting feeding rates and thus liquid height levels in the fermenter, during fermentation (Chenikher et al., 2010; Guez et al., 2007).

Integrated foam fractionation can be applied in different operation modes and adjusted to the foam formation rates in the fermenter, independent of the fermentation mode. Foam separation can be operated continuously or periodically, in cycles of specified durations. In the first operation mode, the generated foam is collected continuously and analyzed at the end of a batch (Bagés-Estopà, 2017; Biniarz et al., 2020). Whereas when operated periodically, the separated foam is collected after distinguished periods (Gao et al., 2018; Zheng et al., 2020). This can be achieved, for instance, by directing the foam sequentially into different foam traps, which can be separated from the foam fractionation setup aseptically (Beuker et al., 2016; Kügler et al., 2015). The latter operation mode becomes convenient for quantification and analysis purposes. For external foam columns, where the foam is generated actively, a distinction in foam generation mode with respect to the gas phase and the liquid phase is made. Here, a batch, semibatch, and continuous operation are possible (Stevenson, 2012).

In foam fractionation systems without recirculation of the foamate, the foamate volume can easily be quantified (Andrade et al., 2017; Barros et al., 2008). In implementations where the foamate is recirculated continuously, only a periodic operation enables adequate analysis and quantification; for instance, via alternate operation of several adsorption columns or foam collecting vessels (Anic et al., 2018).

All previously mentioned operational parameters and the constructive aspects and different technical implementations can contribute to adjusting both enrichment and recovery of each fermentation process. No global design-specific correlation could be concluded for individual substances or product categories in general. However, the occurring effects and mechanisms are universal (Table 1).

5 | FOAM DISRUPTION METHODS APPLIED WITH FOAM FRACTIONATION

When implementing a foam fractionation design, foam disruption may be combined if foam breakage in the foam collecting vessel does not occur independently or fast enough compared to the foam formation velocity in the fermenter. One passive method for foam disruption is the extraction of the foam stabilizing substances from the foam fraction. Thus, foam bubbles are destabilized, and the foam collapses. This method was used by Anic et al. (2018), who installed an adsorption unit packed with C18 silica-based adsorbent to their foam fractionation setup. Rhamnolipids in the foam fraction adsorbed on these particles, which led to surfactant depletion in the foam, resulting in its collapse.

When designing packed adsorption units, clogging may become an issue during fermentation. Therefore, sufficient void space between the adsorbent material particles for biomass and culture broth flow, and an easy subsequent product recovery needs to be considered. This can be achieved by selecting an appropriate adsorbent material with large enough spherical particles, for instance (Anic et al., 2018). Further, it is necessary to consider the pressure resistance of the used vessel materials (Anic et al., 2017). Using an adsorbent unit as a tool for product separation from the foam and for foam disruption is especially suitable if selective adsorption of the

target product can be achieved and if the target product is the only or major substance stabilizing the foam.

Another method for foam disruption in the foam trap is by using traditional chemical antifoam agents, whereas here, the antifoams are applied only in the foam trap and not in the fermenter (Zheng et al., 2015). For instance, Heyd et al. (2011) used citric acid to increase foam disruption during rhamnolipid fermentation. Altering the pH of culture broths containing proteins is generally known to affect foam stability, due to the pH dependence of protein solubility and foam formation capacity (Burghoff, 2012; Vardar-Sukan, 1998). By applying antifoam agents or altering the pH in the foam trap, a chemical foam disruption is possible, without altering the culture composition. However, this is only applicable for implementations where the foam is not recirculated back to the fermenter.

Classical mechanical foam disruption methods include stirring or applying mechanical rotors in the collecting vessel (Alonso & Martin, 2016; Jia et al., 2020). An application of a cyclone to separate the gas phase from the liquid phase was described by Czinkóczky and Németh (2020). Shear stress caused by a packed bed can further be used to rupture foam lamellae (Banerjee, 1993). Küpper et al. (2013) described using a sprinkler unit to sprinkle foamate on the foam in the vessel to increase foam rupture velocity during rhamnolipid fermentation.

Pressure variation may also be used for foam disruption in the foam trap. For example, Gong et al. (2009) showed that applying gas pressure to the foam collecting vessel can be used to liquefy the collected foam during surfactin fermentation. By implementing two sequential foam collecting vessels and applying gas pressure to the second one, they managed to avoid the loss of culture broth due to excessive foam formation without the need to increase the vessel's volume. However, the pressure resistance of the used vessel material is crucial, if high pressure is applied in the foam trap.

The necessity of applying a foam disruption method in combination with foam fractionation is substance- and system-specific and therefore needs to be assessed individually. Adsorptive and chemical methods are substance-specific, whereas mechanical methods are universal and independent of the particular product class. However, biomass and molecule stability toward occurring physical stress need to be evaluated when applying mechanical methods.

6 | PROCESS ADVANTAGES ASSOCIATED WITH FOAM FRACTIONATION

Foam fractionation is usually associated with various process advantages, besides being a solution for excessive foaming. One of the most important benefits of ISPR via foam fractionation is the concentration of highly diluted culture broths, which results in a separated fraction, the foamate, with an enriched product (Davis et al., 2001; Stiefelmaier et al., 2018). This first preseparation step simplifies purification processes at a low cost and low space requirement for additional equipment (Sarachat et al., 2010). The reduced volume necessary to obtain the same amount of target product, due to higher enrichment, results in less energy and time demand (usually needed for separation processes), smaller amounts of salts needed for precipitation (Stevenson, 2012), and smaller chromatography columns, with lower consumption of adsorbent and eluent (Anic et al., 2017). A higher enrichment also avoids competitive adsorption of other media components, when implementing an adsorptive product separation method (Anic et al., 2017). Depending on the used purification units, an optimum between enrichment and recovery rate can be adjusted by varying the operational parameters during foam fractionation (Cui et al., 2014).

Another advantage of ISPR via foam fractionation is evident when product stability is not compatible with process parameters necessary for fermentation, like temperature or pH range. Separating the product continuously enables its preservation without the need to alter the optimal fermentation conditions essential for microbial growth (Stiefelmaier et al., 2018). ISPR via foam fractionation, in some cases, may further increase product yields because separating the product from the broth prevents feedback inhibition and thus stimulates its production (C. Chen et al., 2020; Cooper et al., 1981; Liu et al., 2010; Zheng et al., 2015).

Compared to antifoaming agents, foam fractionation does not cause compromised product functionality or other interactions with the desired product (Atwa et al., 2013; Kottmeier et al., 2012; Winterburn et al., 2011b). It can also be applied when mechanical foam breakers are unfeasible due to limited foam breaking (Kim et al., 1997).

All the previously mentioned advantages associated with foam fractionation can ultimately lead to a facilitated purification process with less equipment demand and a reduction in energy and material consumption. This would mean a reduction in overall production costs and an increase in process sustainability. Nonetheless, foam fractionation is still barely applied in industrial fermentations. This may be due to the various challenges that still need to be overcome to establish foam fractionation on larger scales. Information on scalability is also very scarce. In the following, challenges associated with foam fractionation are discussed further.

7 | CHALLENGES ASSOCIATED WITH FOAM FRACTIONATION

Common challenges present in many foam fractionation systems include the loss of biomass and nutrients and a variety of intersecting process parameters. Furthermore, reduced product activity is typically associated with proteins.

7.1 | Loss of biomass and nutrients

Besides the target molecule, other media components can also accumulate in the foam, entrapped in the liquid layers between gas bubbles. The most often encountered problem when integrating foam fractionation in a fermentation process is the loss of biomass

due to foaming, leading to reduced specific productivity and complicated downstream processing (Alonso & Martin, 2016; D. Zhang et al., 2015). The extent of this issue depends on cell surface hydrophobicity, morphology, and size and is influenced by the media composition. For example, for surfactin fermentation by *Bacillus subtilis*, low cell enrichment of 0.5–1.6 was reported (Alonso & Martin, 2016; Willenbacher et al., 2014), while for rhamnolipids, values of around three were measured (Küpper et al., 2013). This demands process adaptations to minimize cell loss during foaming.

A straightforward, technically simple option is the recirculation of the foamate into the bioreactor, as described in Section 4.1. A general prerequisite for this is the cells' resilience to fluctuations in oxygen supply and shear stress. The recovery of the foam fraction may, however, be reduced by flushing back product in the fermenter. This can be prevented by implementing a fractionation column operated in stripping mode instead of a foam trap and pumping back leached broth instead of foamate, as demonstrated by Winterburn (2011). Another, more product-specific solution is the integration of a product adsorption unit (Anic et al., 2018).

While pumps for recirculating washed-out cells add energy costs to the low-energy ISPR foam fractionation, membrane separation can also reduce biomass loss (C. Chen et al., 2020; Zheng et al., 2020). Membranes tend to be blocked. To ease this, Zheng et al. (2017) constructed a special column comprising a perforated inner column, an enclosing membrane, and an outer column. However, operation conditions can be limited due to specific aeration requirements (Cui et al., 2014).

Biomass immobilization can further be used to reduce biomass loss. The entrapment of *Pseudomonas aeruginosa* in magnetic alginate beads for rhamnolipid production yielded high product enrichment and a stable process that lasted for 3 weeks. Nevertheless, problems typically linked with cell immobilization occurred, for example, cell leakage from the beads and clogging of the channels inside the immobilisates by excessively growing cells (Heyd et al., 2011). Different materials were employed for cell immobilization, which can adversely affect foam formation (Gao et al., 2018; Khondee et al., 2015). For example, chitosan as a carrier material has a foam diminishing effect leading to increased necessary energy input for foam generation. In the case of lipopeptide fermentation, this was one reason for choosing a sequential instead of an integrated foam fractionation (Khondee et al., 2015). Biomass immobilization, in general, is only feasible for secondary metabolites and potentially results in additional maintenance requirements (Heyd et al., 2011). In terms of foam fractionation, the conditions needed for foam generation and the effect of resulting shear forces on the immobilisates have to be considered.

An in-depth understanding of the process, including mechanisms of the substance and cell accumulation in the foam phase and their influencing factors, helps develop tailored solutions. For example, in the case of *Pseudomonas* sp., it is hypothesized that cell surface charge influences cell accumulation in the foam phase, which may be affected by the addition of multivalent anionic ions (Beuker et al., 2016). Blesken et al. (2020) showed that in the case of rhamnolipids fermentation by *Pseudomonas putida*, surface structures are a significant factor promoting cell enrichment. Removing the flagellum and an adhesion protein responsible for cell hydrophobicity by genetic engineering reduced biomass loss by up to 51%.

The extent of cell enrichment during foaming should be quantified for each process as it can significantly decrease productivity. If the cells are sufficiently robust, an implementation of recirculation is the most common measure, which does not need extensive preliminary experiments and process adaptations. No additional energy input is necessary when implementing a membrane for cell retention, but more preliminary experiments have to be conducted, and the long-term stability of the process may be decreased. The same disadvantages arise when using immobilized cells but once established, this solution entails the usual advantages of immobilisates, like higher cell density, increased volumetric production, and simplified downstream processing (Khondee et al., 2015).

Foam separation may also lead to a significant liquid loss of up to 50% (Davis et al., 2001; Salleh et al., 2011). Moreover, substrates, nutrients, and metals can accumulate in the foam (Beuker et al., 2016; Heyd et al., 2011). This issue is described by fewer publications than biomass loss, but is especially relevant for lipopeptides, as they show a high affinity toward divalent metal cations. Fe^{2+}, Mg^{2+}, and Ca^{2+} were enriched up to 8.9 together with the biosurfactants in the foam (Rangarajan & Sen, 2013).

Media components can be replenished by feeding approaches that are only reasonable if the liquid or substrate loss occurs to a small extent. Recirculating the foamate can also be a viable, straightforward solution. Furthermore, the loss of liquid and dissolved molecules is minimized by increasing drainage in the foam phase by technical adaptation, as discussed in Section 4.

7.2 | Reduced product activity

A challenge encountered when foaming enzymes or peptides is the loss of catalytic activity. Three mechanisms are discussed as a reason for that: oxidation by the sparging gas, shear stress caused by bursting gas bubbles, and surface denaturation at the gas–liquid interface. The latter was determined as the main influencing factor and is caused by the orientation of the hydrophobic molecule part toward the gas phase, leading to a change of the tertiary, partly also secondary protein structure (Barackov et al., 2012; Clarkson et al., 1999).

The extent of activity loss due to foaming depends on their general stability and tertiary structure, for example, a low number of disulfide bonds increase the probability of unfolding (Brown et al., 1999). While in the industrial separation of nisin by foam fractionation an activity loss of around 10 % is usual (Stevenson & Li, 2014), this was no issue during lipase and laccase foam fractionation, for example (Gerken et al., 2005; Linke et al., 2007).

Besides protein structure, process conditions such as pH and ionic strength as well as shear stress-inducing parameters (e.g., gas

flow rate, stirrer speed, and foam height) are also decisive for the extent of activity loss (Clarkson et al., 2000; Linke et al., 2007). In general, there are many approaches to enhance enzyme stability, such as those summarized by Iyer and Ananthanarayan (2008). For the foam fractionation of nisin, the addition of trehalose was effective, as this general protein stabilizer reduced the product inactivation from 20 % to 5 % (Kaushik & Bhat, 2003; Y. Wang et al., 2012).

Loss of product activity is further aggravated by additives like cetyl trimethyl ammonium bromide or sodium dodecyl sulfate employed to promote foaming of less surface-active proteins, as they act as enzyme inhibitors (W. Wang et al., 2009). To restore enzyme activity, the use of β-cyclodextrin after foam fractionation proved successful, as it functions as a detergent-stripping agent, allowing the enzyme to renature (Burapatana et al., 2005; M. Zhang et al., 2020).

Foam fractionation is generally considered a gentle purification method for proteins (Linke & Berger, 2011). The findings here underline the importance of considering activity loss during process optimization.

7.3 | Complex process establishment

Another challenge regarding the implementation of foam fractionation in a fermentation process is the complex process establishment. Because foaming is influenced by a variety of interacting factors, for example, gas flow rate, foam fractionation dimensions, and physicochemical parameters, there is no standard guideline on how to design a suitable process. In contrast, it has to be developed for the specific target species. Thereby, process optimization often is a trade-off between enrichment and recovery rate, as demonstrated, for example, by Merz, Burghoff, et al. (2011) for the separation of a fungal cutinase and nicely explained by Burghoff (2012).

It is even challenging to transfer process parameters between similar processes. For example, two extracellular esterases from the fungi *Pleurotus sapidus* showed different foaming behaviors, probably due to their different physicochemical characteristics. So, they could not be separated together, and a two-step foam fractionation process with different pH and detergents was necessary (Linke et al., 2009). Also, preliminary tests in designed culture mixture models for developing suitable foam fractionation conditions only give limited information, as already small changes between the model and the real mixture influence the separation process. This issue of partial transferability and many influencing factors are mirrored in a large number of investigations for parameter optimization (e.g., Brown et al., 1999; Merz, Zorn, et al., 2011; Sarachat et al., 2010).

This general issue regarding foam fractionation is emphasized as soon as it is integrated into the fermentation process. Here, the best conditions for the biocatalytic reaction may differ from the optimal conditions for product separation. For example, during rhamnolipids fermentation by *Pseudomonas putida*, high productivity was reached with high gassing rates, which reduced the product separation efficiency (Blesken et al., 2020). This issue was solved by changing the experimental setup from an integrated internal foam column to an integrated external foam column with a product adsorption unit. In cases where suitable parameters for foaming lead to reduced productivity due to biomass loss, process adaptation as discussed in Section 7.1, may be necessary.

8 | FUTURE PERSPECTIVES AND PROCESS SCALABILITY

Despite the long history and various designs of foam fractionation units, no standard implementation during fermentation has been established yet. This is mainly due to the dependence on various individual factors of each fermentation process that affect its foaming behavior and thus foam fractionation. However, to ensure a successful implementation, some guidelines can be followed when designing a fermentation process with integrated foam fractionation.

The general approach followed by many researchers comprises preliminary studies regarding factors influencing foaming behavior and the empirical determination of the most suitable operating conditions for fermentation and product separation (Rangarajan & Sen, 2013; Winterburn, 2011). These preliminary investigations need to be done for each fermentation system individually due to the limited transferability of results, as previously mentioned. To reduce the number of experimental runs, statistical approaches can be applied (Merz, Burghoff, et al., 2011; Merz, Zorn, et al., 2011).

There are also a variety of approaches to model the foam fractionation process, which showed good agreement with experimental data, but are still very process-specific (Du et al., 2000; Neely et al., 2001). The formulation of good heuristic models is aggravated by the heterogeneity and variety of considered parameters when describing foam fractionation processes and still missing standardized quantification methods and definitions for values such as foamability (Burghoff, 2012; Koop et al., 2020). A more process-independent approach was described by Martin et al. (2010). Here, the process description was reduced to a set of significant dimensionless parameters. Thus, only limited experiments were necessary to describe the surfactant adsorption, foam drainage, bubble size, and mass transfer. This approach simplified experimental studies and process design. Hofmann et al. (2015) followed a similar approach, characterizing foam fractionation of the model system β-casein over the bubble size and linking its influence to other process parameters. The investigation of transferability to other fermentation systems is still pending. Another study describes a theoretical calculation method for a continuous foam fractionation column, including a graphical approach to estimate the theoretical stages (Lemlich, 1968). A theory for predicting the rate of foam overflow, depending on the gas flow rate and the column's cross-section, is also presented there.

The increasing number of publications regarding process development improved the understanding of influencing factors and the foaming process in general, promoting a more knowledge-driven, less empirical process design. This can be accelerated by

applying the design of experiment approaches and mathematical models. The influence of different parameters on product recovery and enrichment presented in Table 1 can further guide the selection of operational parameters and implementation designs. However, more universal parameter definitions and specifications are necessary for additional improvements and simplifications in this area.

Because of the still existing need for preliminary studies, most current research efforts dealing with foam fractionation systems during fermentations are considered in rather small scales. More than 50% of the analyzed fermentations were conducted in fermenters of less than 5 L (Table S1). Few authors presented the first approaches to evaluating process scalability in bioreactors (Bagés-Estopà, 2017; Biniarz et al., 2020; Linke et al., 2009; Winterburn et al., 2011b). Bages-Estopa et al. (2018) reported a successful scale-up from a 1- to a 5-L fermenter, accompanied by a threefold increase in trehalolipid yield, when using a simple foam trap for foam fractionation. Biniarz et al. (2020) demonstrated a scale-up from 3 to 42 L, showing similar foaming behavior in both scales. Only three research groups reported successful fermentations with coupled foam fractionation in a volume larger than 40 L (Barros et al., 2008; Biniarz et al., 2020; Küpper et al., 2013). Fermentation in a 300-L fermenter with 50 L broth volume was described as a biosurfactant (Küpper et al., 2013). However, no information on the scale-up characteristics was given here.

Due to their simple design, foam traps could easily be scaled up. Nevertheless, more research needs to be done to evaluate recirculation behavior and process stability, as well as the possible effect of scale on enrichment and recovery.

For foam columns, one industrial application of nisin fermentation coupled with foam fractionation is reported at the Tianjin Kangyi Biotechnology Company in China. There, a throughput of 30 tons per day is achieved, distributed among eight foam columns (Burghoff, 2012; Stevenson, 2012). This demonstrates the applicability of foam columns on an industrial scale. Crofcheck and Gillette (2003) further showed that the product recovery in a pilot-scale column could be predicted with the recovery measured in a laboratory-scale column, emphasizing the general scalability of foam columns. However, if applied as an integrated internal foam column, further constructive considerations concerning the reactor and column dimensions need to be taken into account. For an integrated external foam column application, on the other hand, constructive and dimensional considerations become less severe. A parallel operation of several smaller columns becomes a feasible scale-up solution.

When discussing scalability, long-term process stability is a crucial prerequisite. In the analyzed studies, the duration of fermentation coupled with integrated foam fractionation varied between a couple of hours up to 500 h of continuous operation. ISPR via foam fractionation, coupled with immobilized biomass, enabled the continuous rhamnolipid fermentation for 500 h (Heyd et al., 2011). Foam fractionation, especially if combined with recirculation, can usually avoid or at least minimize a severe loss in culture broth due to overfoaming. This was shown by Küpper et al. (2013), where applying foam fractionation enabled an increase in fermentation duration from 1 to 2 h to a couple of weeks. Nevertheless, the positive effect of foam fractionation on increasing fermentation duration is only given if foam formation can be controlled and process stability is achieved. This makes a proper understanding of parameters influencing foam formation even more crucial for a successful implementation.

For future applications, a critical evaluation of process economy would help to better assess the foam fractionation coupled processes, especially compared to conventional fermentation. In general, it can be assumed that foam fractionation would result in a better overall process economy due to the simplified proceeding purification process and savings in anti-foam agents or other foam rupture apparatuses. An increase in the ecological impact of the process coupled with foam fractionation, compared to the traditional one, is at the same time not expected. On the contrary, the potential savings in energy and solvents during the proceeding purification should result in a more ecological process.

9 | CONCLUSION

The complexity of different parameters affecting individual fermentation systems still hinders the development of one universal foam fractionation design. For initial investigations and simple implementations, the basic concept consisting of a foam trap presents a sufficient solution. Foam columns serve as a flexible tool to adjust product enrichment and recovery. But the main challenge remains the process establishment, as it is a trade-off between biological and technical considerations, enrichment, and recovery. More research regarding process-independent mathematical models and a uniform recording and assessment of empirical parameters may improve this and ultimately contribute to a widespread use of foam fractionation at larger scales.

AUTHOR CONTRIBUTIONS

Amira Oraby: Conceptualization; formal analysis; investigation; methodology; visualization; validation; writing—original draft. **Isabell Weickardt**: Data curation; formal analysis; investigation; visualization; writing—original draft. **Susanne Zibek**: Funding acquisition; resources; supervision; writing—review and editing.

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DATA AVAILABILITY STATEMENT

All data used to support the findings of this systematic review are referenced in the manuscript and the supplementary document.

ORCID

Amira Oraby http://orcid.org/0000-0002-1001-8082
Susanne Zibek http://orcid.org/0000-0001-5344-6549

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