Application of rotating packed bed for in-line aroma stripping from cell slurry

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Abstract

BACKGROUND: Nowadays, biotechnological production receives increasing interest as an alternative source of natural aromas. Unfortunately, especially for hydrophobic and semi-volatile aromas, the heterogeneous product partitioning between all phases present in fermentation makes recovery challenging. Additionally, when an aroma displays an inhibitory effect on the production microorganism, product removal during fermentation is recommendable. In-line aroma stripping offers an elegant way to deal with such challenges. This study reports the use of rotating packed bed (RPB) technology for the intensification of stripping of α-ionone, a key aroma of raspberry, from a model fermentation slurry containing *Saccharomyces cerevisiae* cells in a concentration of 250 g-CWW L^{-1}.

RESULTS: Throughout all experimental investigations, yeast cells were robust towards both the chemical stress from aroma exposure at a concentration of up to 400 mg L^{-1} and the mechanical stress from peripheral equipment and rotation of up to 2750 rpm, as a maximum of 11.3 ± 0.5% disrupted cells were measured during continuous processing in an RPB. An increase in the rotation speed led to an enhanced transfer of α-ionone from the fermentation slurry to the gaseous phase.

CONCLUSIONS: RPB technology is found to be promising for the intensification of in-line stripping of biotechnologically produced aromas from crude fermentation broth without cell separation. The use of subsequent RPBs equipped with custom packings and flexibly adjustable rotation speed displays a holistic aroma recovery process supporting the way to commercial competitiveness of biotechnological aromas.

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Keywords: rotating packed bed; natural aromas; in-line stripping; downstream processing; cell viability

NOMENCLATURE

$c_{\alpha-ionone,sol}^{aq}$	aqueous solubility concentration of α-ionone [$g_{aroma} L_{water}^{-1}$]
$c_{\alpha-ionone}^{aq}$	measured aqueous concentration of α-ionone [$g_{aroma} L_{supernatant}^{-1}$]
c	total concentration of α-ionone [$g_{aroma} L_{suspension}^{-1}$]
d_i	inner diameter of packing used in RPB [m]
d_o	outer diameter of packing used in RPB [m]
$\dot{G}$	gas flow rate [L min^{-1}]
h	height of packing used in RPB [m]
$\dot{L}$	liquid flow rate [L min^{-1}]
$\log K_{ow}$	water–octanol partition coefficient
S_0	gas sensor baseline value [%]

INTRODUCTION

Aromatic compounds have been valued and widely used as parts of flavors and fragrances for centuries. Nowadays, the increasing application of aromas in such products as foods, feeds, household items, toiletries and even pharmaceuticals boosts the development of novel production techniques.[1] Recently, increasing consumer call for greenness, naturalness and sustainability, combined with the legal framework, has led to progress in the use of biotechnology for aroma production as an alternative to natural extraction and chemical synthesis.[2–4] Mild reaction conditions, no toxic waste, high enantioselectivity, predominantly renewable feedstock and natural labeling are some of the advantages of biotechnological aroma production to name a few.[4,5] On the contrary, the recovery of biotechnologically produced compounds from complex, solid-containing broths is challenging. Dealing with aromas, heterogeneous phase partitioning, product volatility and cell toxicity are some major hurdles to overcome in order to reach commercial competitiveness.[6,7] Water-immiscible solvents for a two-phase fermentation or in-line product extraction are often applied.[8] However, even when green and sustainable solvents are used, their application is still challenging because of safety issues, and general toxicity to cells for such solvents with $\log K_{ow}$ values lower than five.[9] One possible

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alternative for product recovery from all phases simultaneously, preventing growth inhibitory effects and shifting the equilibrium towards product formation, is the stripping of aromas from a bioreactor during production. When stripping is done *in situ* directly in the bioreactor, the choice of stripping gas and the adjustment of the gas flow rate and/or gas temperature are constrained by the fermentation. One possible way to keep the process flexible and decouple aeration rate and stripping rate is the use of in-line stripping in an external process loop. In order to protect downstream apparatuses from blocking and fouling, biomass retention is required. However, if the aroma compound is found in or on the cells, stripping from biomass also becomes necessary. Here, a stripping apparatus is required, which, on the one hand, could increase the stripping of aroma from cell slurry, transferring more aroma into the gaseous phase, especially for semi-volatile aromas with low vapor pressure. On the other hand, such an apparatus should be able to handle solids without damaging the cells. A rotating packed bed (RPB) presents a promising alternative for the in-line stripping of aromas from a crude fermentation broth, in which an increase of stripping by rotation is combined with bearable mechanical stress on cells.

RPB technology comes from the area of process intensification in a high-gravity field, and RPB devices have been intensively investigated in academia for heat and mass transfer enhancement ever since their use was heavily promoted by Ramshaw in the 1980s.[10] In the last few decades, some successful industrial applications of RPBs for distillation, absorption, stripping and chemical reactions have been reported, best summarized in recent reviews of Zhao *et al.*,[11] Neumann *et al.*[12] and Wang *et al.*[13] The centrifugal field inside the rotating packing of an RPB leads to a more elaborated liquid distribution and the generation of thin films and smaller droplets which are the primary reasons for increased mass transfer coefficients and mass transfer areas.[10,14–16] Besides mass transfer-enhancing characteristics, short residence times of the phases, easy start-up and shut-down and large hydrodynamic operational windows make RPBs more flexible[17–19] and particularly beneficial for processing fermentation broths. Because of its compact design, especially a laboratory-scale RPB could be equipped with customized 3D printed packings adjusted to the process needs.[20] For this study, a spiral packing was designed based on the theory of MacInnes *et al.*[21] with the proposed parameter restrictions to deliver a laminar and segregated flow of both phases with a stable interface. Thus, it offers precise control over the surface area generated as the liquid forms a film flow along the wall of the spiral. In this packing, the liquid film flow is laminar with no disruptive obstacles like in mesh or foam packings, offering a gentle environment for processing living microorganisms. An additional advantage of the spiral packing design combined with the high rotation speed of an RPB is the reduction of the liquid film thickness. According to commonly accepted theories on mass transfer, two-film theory[22] and penetration theory,[23] the generation of thinner films increases the mass transfer of a compound as the diffusion resistance and the diffusion time in the liquid film decrease. To our best knowledge, no applications of RPBs for the continuous gas–liquid processing of crude fermentation broth have been reported so far. The current study presents the experimental investigation of aroma stripping from microorganism-containing slurry in an RPB equipped with a 3D printed spiral packing.

As a model aroma for the investigation, α-ionone, a key flavor of raspberry[24–26] and blackberry,[27] was chosen. This compound can be produced biotechnologically via fermentation of genetically modified fungi like *Yarowia lipolytica*, or bacteria like *Escherichia coli*.[28–30] In previous research, we discovered that during the fermentation of *E. coli*, hydrophobic and semi-volatile α-ionone is found predominantly in or on the biomass, with some amount dissolved in the fermentation supernatant and only a minor fraction found in the gas phase.[31] For such particular partitioning, the use of a mass transfer-enhancing device capable of handling fermentation slurry like an RPB for in-line product stripping seems particularly beneficial. An inevitable process requirement for such an application is to guarantee cell viability, as product formation needs a cellular metabolism. In the study reported here, the influence of chemical and mechanical stress on cell disruption was investigated using an aqueous suspension of *Saccharomyces cerevisiae* mixed with α-ionone. Baker's yeast was taken as a model microorganism from a fungi class because of its generous availability and general safety. Unlike in previous research on α-ionone fermentation and recovery,[31] the microorganism used for the present study was not genetically modified to produce α-ionone. Therefore, the aroma was added to the baker's yeast cell suspension externally. Also, the viability of the cells during continuous stripping over 2 h was analyzed. In order to investigate the influence of increased acceleration on the stripping of aroma from the cell slurry, the experiments were performed at varying rotation speeds. Although the stripping might be increased by elevated gas flow rate and/or gas temperature increasing the volatility of the product, both parameters were kept constant, as the main focus of this study was the evaluation of the RPB performance.

In the subsequent sections, first, the slurry preparation and the general laboratory setup, including the machinery used, are presented. Then a description of the experimental procedure and the analysis techniques follows. The experimental results and a discussion are presented, before the final section concludes the article together with an indication of future research directions.

MATERIALS AND METHODS

Experimental setup

An aqueous cell suspension was prepared from baker's yeast (*S. cerevisiae*) in pure tap water, containing 250 g cell wet weight (CWW) L^{-1} (85 g cell dry weight (CDW) L^{-1} under the assumption of 66% water content[32]) similar to the cell content of an *E.coli* fermentation broth for production of natural α-ionone previously studied.[31] The stripping experiment was set up according to Fig. 1. The suspension was fed from a liquid tank into the RPB using a peristaltic pump (AUTOCLUDE Model V L, South Ockendon, UK) and was distributed at a constant flow rate of $\dot{L} = 0.56$ L min^{-1} axially at the eye of the RPB by an open pipe with 1/8″ orifice. The liquid reflux was collected in the same liquid tank. The supply air was passed through a humidifier and then fed counter-currently into the RPB through the outer edge of the casing towards the eye, leaving the RPB from the top cover. The air flow rate was kept constant at $\dot{G} = 100$ L min^{-1} measured with a TSI 4040 flowmeter (Driesen + Kern GmbH, Bad Bramsted, Germany) with a precision of ± 0.05 L min^{-1}. The temperature of the gas inlet stream was kept constant at 21 ± 0.1 °C. The temperature increase at the gas outlet was measured and did not exceed 1 K. The cell suspension was kept at room temperature of around 21 °C, measured by a glass thermometer (Ludwig Schneider GmbH & Co. KG, Wertheim, Germany) with a scale of ± 1 °C. The RPB used in this study was equipped with a bowl-type perforated rotor (Fig. 2), containing a 3D printed spiral packing (Fig. 3) of
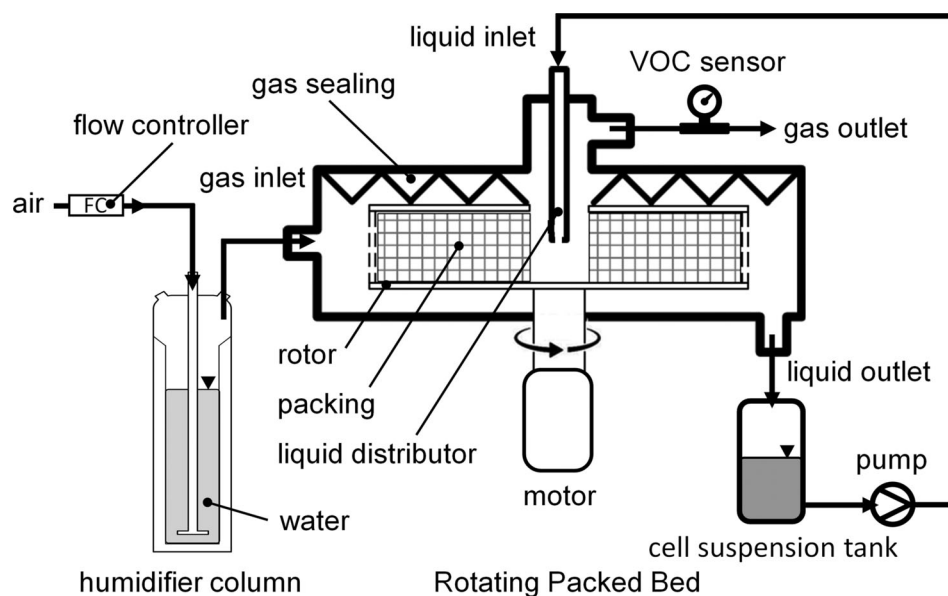
Figure 1 Process diagram of experimental setup for aroma absorption in RPB.

$d_i \times d_o \times h = 45\,mm \times 174\,mm \times 20\,mm$ with a channel width of 2.14 mm designed based on the theory of MacInnes et al.[21] The packing was printed using Formlabs Standard Clear resin,[33] as the studied chemical system is not chemically aggressive. The rotation of the RPB was varied from 500 rpm, equal to 6 relative centrifugal force (rcf) at the inner and 25 rcf at the outer radius of the packing, up to 2750 rpm, equal to 191 rcf at the inner and 741 rcf at the outer radius of the packing. Each experiment was performed in duplicate with a new yeast suspension prepared.

As some aroma compounds, e.g. α-ionone,[29] are known to have an inhibitory effect on microorganism growth, the effect of aroma addition on cell viability was investigated. Therefore, the yeast cell suspension was exposed to α-ionone at a concentration of $c = 400\,mg\,L^{-1}$ equal to the maximum product titer reported in a previous study of Lukin et al.[31] and around four times larger than aqueous solubility of $c^{aq}_{\alpha-ionone,sol} = 105.7 \pm 3.7\,mg\,L^{-1}$ (data for 25 °C).[34] In order to determine whether an increased exposure time increases the cell damage, the cell suspension was incubated with

α-ionone for different times of 5, 10, 20, 30 and 60 min and up 20 h. The viability of cell samples was analyzed and compared to samples of the native cell suspension incubated under identical conditions for equal times without aroma addition (negative control). In order to investigate the influence of the mechanical stress on the viability of cells during processing in the RPB, the cell suspension was pumped for 30 min in a closed loop through the open pipe liquid distributor dismounted from the RPB. Afterward, cell samples were analyzed and compared to the native suspension without pumping (negative control). Furthermore, the influence of different rotation speeds on cell viability in an RPB equipped with a bowl-type perforated rotor was investigated.

For the stripping experiments, native yeast suspension was first pumped through the RPB in a closed loop for 1 h at 500 rpm to

Figure 2 Perforated bowl-type rotor used in RPB.

Figure 3 3D printed spiral packing used in the experimental study.

condition the gas sensor to the yeast volatiles. After the gas sensor was zeroed to its baseline of $10 \pm 1\%$ and a stable signal was ensured for 10 min, α-ionone was added manually to the suspension tank at a concentration of $c = 400$ mg L^{-1}. The rotation speed of the RPB was increased stepwise from 500 to 1500 rpm and then to 2750 rpm, ensuring a constant gas sensor signal for 5 min in each step. During each step, samples of the cell suspension were taken. For the measurements of time-dependent stripping behavior, the rotation speed was kept constant at 1500 rpm for 2 h, and cell suspension samples were taken at discrete times for the measurement of cell viability at the liquid outlet of the RPB. In this manner, unlike in the procedure discussed above, the effects of both chemical and mechanical stresses on cell viability were investigated together. This allows not only conclusions to be drawn about the single-parameter effects but also provides an estimate for the interaction effect of the increased stress. α-Ionone phase partitioning was determined in an independent experimental setup similar to the stripping experiment described above. Samples of yeast slurry were collected at the liquid outlet of the RPB 3 and 30 min after the beginning of the stripping and α-ionone concentration in the separated aqueous and solid phases of the slurry was analyzed using high-performance liquid chromatography (HPLC) with diode array detection.

Analytics

Cell viability was analyzed optically using a light microscope (Bresser Science TRM 301, Rhede, Germany) at an overall 400 times optical magnification. Cell suspension samples were diluted with tap water in a ratio of 1:1000. A total of 20 μL of the dilution was placed on a microscope slide and mixed with a drop of 0.5% methylene blue solution. The number of native (vital) and dyed (disrupted) cells was counted immediately after mixing, as contact with methylene blue damages all cells after a maximum of 5 min. In order to gain a statistical accuracy, 4 B-fields containing 16 C-fields each were evaluated, and average numbers were calculated for one B-field. The evaluation was done based on the percentage of the dyed (disrupted) cells as a measure for the negative influence of different stress conditions during processing in the RPB on cell viability (Fig. 4).

For the measurement of α-ionone concentration in the yeast slurry, 30 mL of sample was centrifuged in a laboratory centrifuge (Centrifuge 5804 R, Eppendorf, Germany) at 4500 rpm (3622 rcf) at 25 °C for 5 min to separate the phases. The solid phase was re-suspended with 0.25 g_{water} g_{solids}^{-1} in deionized water to prevent cell clogging during solvent extraction. The aroma was extracted from yeast cells with n-hexane at $\varphi = 0.4$ $g_{solvent}$ g_{solids}^{-1} for 1.5 h in an overhead shaker at room temperature, similar to the method previously described by Lukin et $al.$[31] In total, three extraction steps were performed, with fresh solvent used in every step. In order to minimize the aroma loss between the handling steps, the samples were sealed as tightly as possible, and the transfers were performed as fast as possible. However, it is inevitable that some aroma will partition in the headspace of the equipment used. All experiments were measured in duplicate with the error indicating the standard deviation between the experiments. The concentration of α-ionone in the solvent and in the aqueous phase was determined using HPLC analysis (Knauer, Germany) with a NUCLEODUR™ 250x4 C8ec column (Macherey-Nagel, Germany). Acetonitrile (ACN; HiPerSolv CHROMANORM®, VWR International, USA) and Millipore water were used for elution. The solvents were mixed with 1% (v/v) acetic acid (GPR Rectapur 99–100%, VWR Chemicals, USA) and stored separately. The oven

temperature was 40 °C and the following gradient was applied: starting at 60% ACN isocratic for 4 min to 100% ACN gradient for 4 min to 100% ACN isocratic for 1 min and finally to 60% ACN equilibration step for 6 min. The concentration was measured using a diode array detector at 245 nm (Knauer, Germany) according to prior calibration with analytical standard α-ionone (Sigma-Aldrich/Merck, Germany).

The aroma concentration in the gaseous stream was measured by a semiconductor-based mix gas sensor (VOC-A-UI, ConSens GmbH, Ilmenau, Germany) used for monitoring compartment air quality. Although this sensor is not designed and is not explicitly applied for the measurement of aroma concentration in gaseous streams, it presents a fast, robust and low-cost way for the real-time online analysis of RPB performance. As the volatile organic compound (VOC) sensor signal is linearly proportional to the concentration of the given aroma compound (data not shown), and the system was operated with only one aroma at a time, the sensor value can be taken directly as a qualitative measure without any concentration calibration. Besides, this study is primarily aimed at proving the principle of RPB use for the enhancement of aroma stripping without cell damage. Therefore, the evaluation of stripping enhancement based on the gas sensor signal would lead to the same qualitative outcomes as based on the gaseous aroma concentrations. In order to subtract the influence of the native yeast volatiles, the baseline of the VOC sensor ($S_0 = 10 \pm 1\%$) was zeroed to the pure yeast suspension for the respective experimental conditions. First, without any α-ionone addition, the cell suspension was pumped in recycle through the RPB at the given gas and liquid flow rates. After the signal of the VOC sensor had remained in steady state for 10 min, the rotation speed was increased from 500 to 1500 rpm for 5 min and then to 2750 rpm for another 5 min. The change of the steady-state signal value remained within $\pm 2\%$ between the changes of the rotation speed, meaning that no more yeast volatiles were stripped at the increased rotation speed. Then the rotation speed was set to 500 rpm again, and the VOC sensor was zeroed after 5 min of constant signal value to the baseline of $S_0 = 10 \pm 1\%$. After ensuring a constant signal for another 10 min, α-ionone was added to the suspension, and the VOC sensor signal time evolution was measured.

RESULTS AND DISCUSSION

A critical process requirement for in-line product recovery is to ensure the viability of the cells, as in most fermentation processes product formation is coupled to the metabolism of living cells. Although the concentration of the aroma compound should be kept a priory below an inhibitory value, here, the influence of chemical stress was investigated to separate this effect from the effect of mechanical stress. Most of the mechanical stress is expected from the pump and the liquid distributor, primarily because the narrow pipe could act as a pressure homogenizer. Therefore, cell viability after the exposure to α-ionone and after pumping through the liquid distributor was analyzed and compared to negative control. Because the cell viability did not vary much with exposure time to α-ionone, in Fig. 5 only the measurements after 5 min and 20 h are shown.

Notably, no disrupted cells were detected either in the negative control or in the cell suspension incubated with α-ionone for 5 min, and not in the suspension passed through the pump and the liquid distributor. Only in the samples exposed to the aroma for 20 h were just a few single disrupted cells observed in only
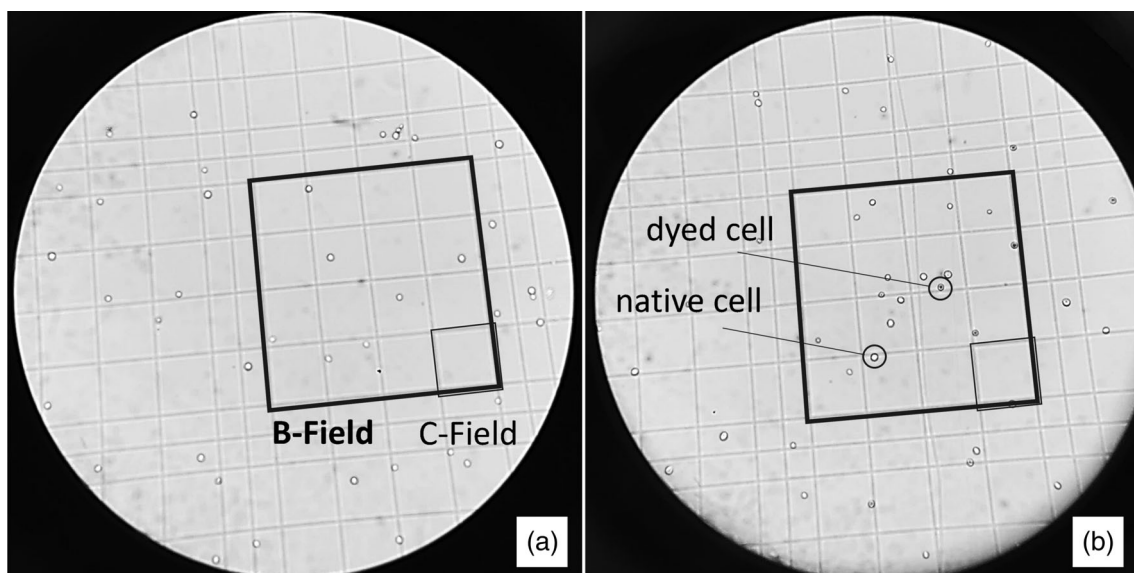
Figure 4 Typical microscopic picture of yeast suspension samples mixed with 0.5% methylene blue: (a) negative control; (b) continuous α-ionone stripping for 60 min.

some of the B-fields corresponding to 2 ± 4% of total cells. Considering the error and with the observation of a more narrow time screening of the samples (data not shown), it can be concluded that the yeast cells survive the exposure to the chemical and mechanical stress levels applied in this study arising from aroma addition and peripheral equipment. In addition to the liquid distributor, small perforations of the rotor (Fig. 2) combined with high rotation rate could also damage the cells. In Fig. 6, the proportion of disrupted cells is presented as a function of the rotation speed applied. A value of 0 rpm stands here for the negative control of the cell suspension, which was not fed through the RPB.

Also here, no crucial effect of the rotation on cell viability is observed. Only at the highest rotation used was a slight increase in the disrupted cells observed. However, considering the error bars, indicating the standard deviation of the experimental duplicate, this increase is statistically not significant. Although the error

bars are displayed symmetrically, the deviations in the proportion of native cells larger than 100% are physically meaningless. Despite even when considering only one-sided negative error representing a worst-case scenario, the differences in the native and disrupted cell percentage between any rotation speeds investigated are statistically not significant. Therefore, there is no statistical evidence that the increase in rotation speed has any influence on the number of disrupted cells. The results show that under the conditions applied here the cells survive the processing in the RPB even at increased rotation speed and even when a rotor with small perforations is used. Therefore, in-line stripping of α-ionone from the cell slurry can be done in the current RPB without any negative effect on cell viability.

Figure 7 displays both the effect of rotation speed on stripping and the time evolution of gaseous aroma concentration during the continuous stripping of α-ionone from the cell suspension. Also, the time-dependent cell viability is presented as the percentage of disrupted cells in the background in order to estimate the yeast stability towards prolonged mechanical stress.
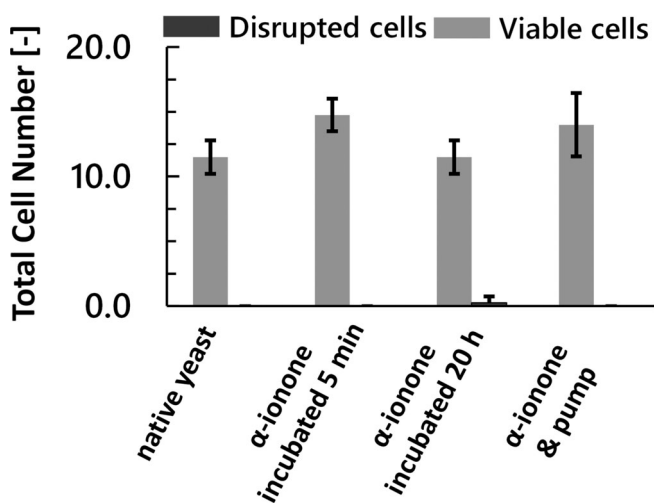
Figure 5 Total cell number for viable and disrupted cells after exposure to α-ionone and processing through a pump with liquid distributor of the RPB compared with native yeast suspension.
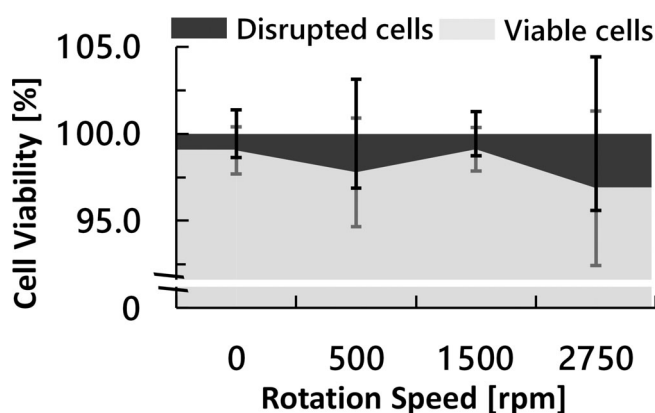
Figure 6 Influence of rotation speed applied in RPB equipped with perforated rotor only on cell viability.
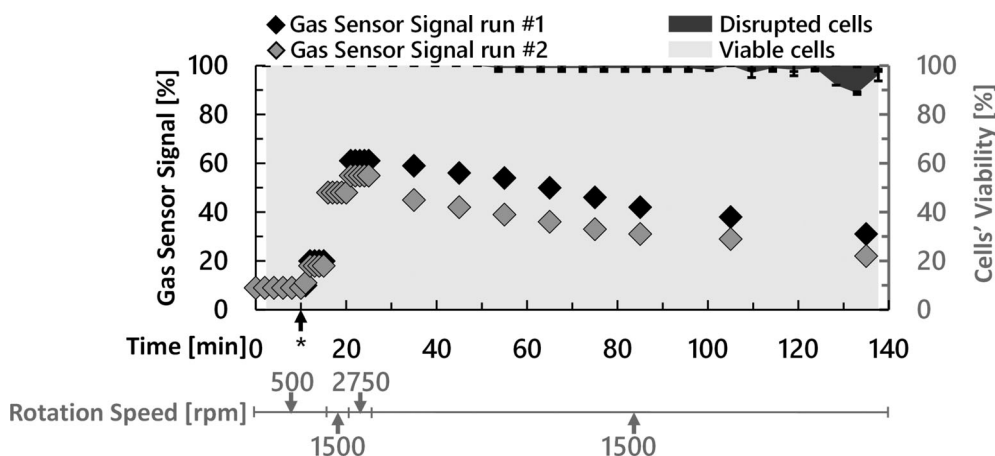
Figure 7 Time evolution of gas sensor signal and cell viability during in-line stripping of α-ionone from aqueous yeast suspension in RPB at different rotation speeds (asterisk marks the addition of α-ionone).

From the data obtained, it can be seen that an increase of the rotation speed leads to an increased stripping as the gas sensor signal increases from 20% at 500 rpm to over 50% at 1500 rpm and up to 60% at 2750 rpm meaning more α-ionone is transferred to the gaseous phase. Thus, an increasing rotation rate has a mass transfer-enhancing effect, which might be traced back to the formation of thinner wall films at higher rotation rate, decreasing the mass transfer resistance. Besides the mass transfer in the packing of the RPB, the droplets formed in the cavity zone between the rotor and the casing of the machine and the liquid films covering the casing walls can also contribute to the mass transfer by providing an additional mass transfer area. Over the processing time of 135 min at a constant rotation speed of 1500 rpm, the gas sensor signal decreased continuously. As more and more aroma initially added is stripped from the slurry and is discharged from the system, the concentration in the slurry decreases, also lowering the concentration in the gas phase, which leads to a decreased sensor response. Because no aroma was added to the slurry processed in the recycle, and no aroma is produced by the cells, no steady state can be achieved in the presented setup. During the entire experiment, just a small number of disrupted cells were observed, meaning that the cells were also resistant to prolonged mechanical stress of rotating equipment. The notable resistance to mechanical stress could be a result of a rigid cell structure of *S. cerevisiae*. Fungal cells have not only a lipid-based cell membrane but also a cell wall made from polysaccharides, chitin and some proteins, making them more robust.[35] Thus, the use of fungal cells for aroma fermentation could be well combined with in-line product stripping in an RPB for prolonged processing time.

An analysis of slurry samples collected in an independent stripping experiment demonstrates the time development of extracted α-ionone mass and aroma phase partitioning (Fig. 8). Because from the process point of view it is of great interest to know from which phase the most product mass can be recovered, the evaluation was done here based on the mass and not on the phase-specific concentration. By reason of a constant sample size of 30 mL containing 22 ± 2% (w/w) wet solids, the obtained conclusions are also applicable to the phase concentrations. Looking at the samples in total, the mass of aroma extracted from the slurry after 30 min of stripping decreased compared to that after 3 min. As expected from gas-phase monitoring, the aroma is

stripped from the yeast slurry and is discharged from the system with the gaseous phase. Considering the solid and liquid phases separately, the mass of α-ionone found in the aqueous supernatant remained constant also after 30 min of the experiment acknowledging the large standard deviation. The aroma stripped from the aqueous phase is most likely replaced by the molecules desorbing from the solid phase. On the contrary, the mass of α-ionone found in the solid phase decreases during the progress of the experiment. Altogether, the data support the hypothesis that α-ionone is stripped from both phases simultaneously, but at the same time partitions from the biomass into the aqueous supernatant. Thus, the presented system displays a simultaneous three-phase mass transfer of aroma compound: (1) from the solid phase (biomass) into the gaseous phase (air), (2) from the solid phase into the aqueous phase and (3) from the aqueous phase into the gaseous phase. For this system, the determination of the dominant phase resistance would require further bi-phasic mass transfer investigations. Unfortunately, without any stripping from the dry biomass directly into the gaseous phase and without any aroma dissolution from the dry biomass into the aqueous phase only, it is not possible to identify the main mass transfer resistance.

Measurements of a sample taken after 3 min confirm that because of the low aqueous solubility $c^{aq}_{\alpha-ionone,sol} = 105.7 \pm 3.7$ mg L^{-1} (data for 25 °C),[34] most of the added α-ionone adheres
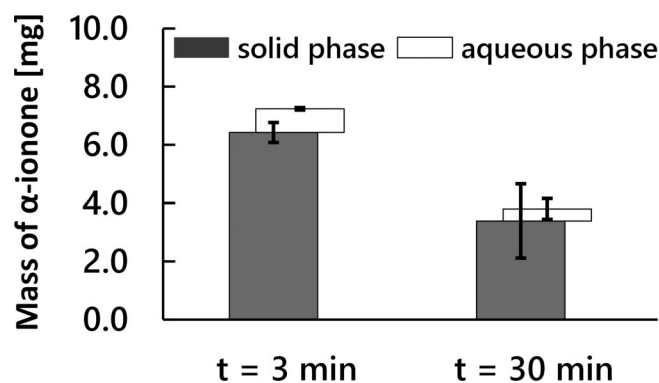
Figure 8 Distribution of α-ionone between different phases of yeast slurry during aroma stripping.

to the biomass and only a small portion is dissolved in the aqueous supernatant ($c_{\alpha-ionone}^{aq} = 36.5 \pm 4.8$ mg L^{-1}). Although the aqueous supernatant represents around $78 \pm 2\%$ (w/w) of the yeast slurry, it contains only $12 \pm 0.1\%$ (w/w) of the overall extracted aroma, showing a preferable α-ionone distribution to the solid phase similar to that in *E. coli* fermentation broth previously reported.[31] Nevertheless, in order to gain the yield maximized aroma recovery, α-ionone can be, for example, adsorbed from the aqueous phase using Diaion HP20 followed by desorption with *n*-hexane.[31]

DISCUSSION

An increasing interest in biotechnological aroma production fuels the development of novel product recovery techniques. In-line aroma stripping from fermentation slurry in an RPB presents a promising technology for the intensification of downstream processes of biotechnologically produced aromas. The rotation of the RPB leads to an increased mass transfer of semi-volatile compounds like α-ionone, enhancing the stripping. This study reports the use of an RPB for in-line aroma stripping from a crude broth using a model fermentation slurry containing 250 g-CWW L^{-1} baker's yeast (*S. cerevisiae*) mixed with 400 mg L^{-1} α-ionone. On the basis of the data obtained from the specific experiments of this study, the following conclusions can be drawn. The yeast is robust to both the chemical stress of exposure to α-ionone and the prolonged mechanical stress arising from the rotating equipment under the conditions applied, making possible in-line operation without cell separation. In the RPB, the increased rotation led to an intensified stripping of aroma from the solid phase as more aroma was transferred to the gaseous phase at the otherwise constant conditions. In order to efficiently recover the gaseous aroma stripped from the slurry in a more convenient form, a second RPB can be used for the intensification of aroma absorption in, for example, plant oil. The independently adjustable rotations in both RPBs and the use of customized 3D printed packings designed for a specific challenge like handling of solids or viscous oil-based absorbents would bring more flexibility into the processes. The rigid cell structure of yeast makes it a preferable host for biotechnological aroma production when in-line stripping using an RPB is considered as a possibility for the intensification of aroma recovery. The transferability of the results to, for example, bacterial cells should be investigated before an RPB application. Furthermore, a more detailed screening of the influence of such parameters like stripping gas, fluid flow rates, aroma concentration and cell dry weight on cell viability and stripping performance is recommended. Besides, in order to get stronger statistically verified results, a much greater number of experimental evaluations should be done. Finally, product stripping from a real fermentation broth should be performed as such a broth contains salts and other components, which could influence both the thermodynamics and the mass transfer of the aroma. Altogether, an RPB has been shown to be a promising tool for the intensification of in-line stripping of natural aromas from fermentation broth.

ACKNOWLEDGEMENTS

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