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Improvement and Modelling of Hexenal Transfer in Liquid-Gas Reactor

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Abstract The aim of the present work is to improve the extraction of an aromatic compound, 2E-hexenal, from a continuous liquid–gas reactor. Having an improved process to recover hexenal could be of interest to obtain this chemical if produced by any biotechnological process. The experimental program proposed on the basis of a full 2^3 factorial design demonstrated that the conditions optima for the extraction are 40°C as a reactor temperature and −10°C as a temperature of trap for a nitrogen flow of 45 mL s^{-1}. These conditions allowed to recover more than 75% of hexenal, when this compound was stripping by a nitrogen stream from the liquid phase. The mass transfer was theoretically and experimentally studied. The model predictions were validated against experimental results obtained for a reactor, and good correlation was observed for a simulation.

Keywords Extraction · Factorial design · Green note · Mass transfer coefficient · Mathematical modeling · Reactor

Introduction

Hexenal in its different isomer forms is a key organoleptic element of green note that is found in both fragrance and flavors. The 2E-hexenal which is commonly called leaf aldehyde is widely distributed in fresh leaves, vegetables, and fruits. The traditional sources of aroma compounds such as plant essential oils cannot give sufficient quantities of hexenal. This motivated research efforts toward finding alternative natural ways that can satisfy the increasing demand of this compound. Flavor and aroma can be derived from a variety of metabolic pathways in plant system. For example hexenal isomers, mainly 3Z-hexenal and 2E-hexenal, are organoleptically known to possess "green character", and therefore, are of commercial interest. They are formed through the lipoxygenase pathway [1, 2] in which linolenic acid (C 18:3) is converted by the sequential action of lipoxygenase

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and hydroperoxide-lyase into aldehydes. Schade et al. [3] used a crude enzyme preparation from tomato fruit to produce hexanal in a packed-bed reactor coupled to a hollow fiber system. In industrial applications of immobilized enzymatic reactions, this kind of reactor is most often used as a continuous operating system because it is easily automated and controlled, and its operating costs are low [4]. In general, to acquire quantitative and qualitative information regarding the volatile compounds obtained after a synthetic step, it was necessary first to isolate the volatile aroma constituents from the involatile bulk of the medium by distillation [5] or head-space condensation [6]. A configuration that has received much attention in recent years is the two-phase partitioning bioreactor. The partition of both reactant and product between the two phases has several advantages compared with monophasic systems. With this configuration, enzyme inhibition by the substrate and/or the product may be smaller; also, the amount of water can be reduced to a level that is just enough to saturate the organic solvent and maintain the activity of the enzyme. A liquid–gas biphasic system is present also in many applications which involve gassy substrate and/or product [7]. Herman et al. [7] have characterized the mass transfer properties of shaken microtiter plates. In this study, the maximum oxygen transfer capacity, the specific mass transfer area, and the mass transfer coefficient in a single well were determined. In the liquid–gas biphasic system, the gas may bubble up through the liquid without being circulated by a mechanical mixer.

The mathematical modeling of biological systems is important for understanding their complex behavior. The purpose of this paper is to present a model for describing hexenal transfer in liquid–gas reactor. This reactor can be applied in biocatalytic conversion using the enzymes of the lipoxygenase pathway to produce hexenal [8–10]. A 2^3 factorial design was used for choosing different parameters (nitrogen flow, temperature of the reactor, and temperature of trap) and to find out their influence on the hexenal transfer. To evaluate the liquid–gas mass transfer, a detailed mathematical model for the heterogeneous system was developed.

Materials and Methods

Liquid–Gas Reactor Set-Up

A continuous reactor for the extraction of hexenal: 2E-hexenal (Sigma, France) has been developed. This reactor was equipped with a magnetic stirrer, a thermometer, a pH electrode, an inlet and outlet of gas. A bath was used to maintain and adjust the temperature of the reactor. In the hermetic reactor, the extraction was achieved by passing nitrogen via the inlet which bubbled up through the reactor containing 0.8 mM of hexenal in glycine buffer pH 9. The hexenal was then removed with nitrogen through the outlet and finally trapped in bath controlled at −10°C.

Improvement of the Hexenal Extraction: 2^3 Factorial Design

The improvement was achieved with a full 2^3 factorial design after an appropriate choice of the most important variables implied in the transport phenomena of hexenal in liquid–gas reactor. Application of this method requires the adequate selection of factors and levels. Three quantitative parameters were studied (nitrogen flow, reactor temperature, and trap temperature), and their effects on a response were investigated. Each factor was present at two levels, + and −, which were used to represent the low and high levels of the factors,

Table 1 Experimental field.

Factors	Nitrogen flow (X1; mL s^{-1})	Temperature of extraction (X2; °C)	Temperature of trap (X3; °C)
Level −	45	20	−10
Level +	90	40	−160

respectively (Table 1). Selection of factors was based on the operating conditions that have a significant influence on the transfer of hexenal in the reactor, and selection of levels was carried out based on results obtained in preliminary studies. Eight runs representing treatment combinations are shown in the design matrix as in Table 2.

Study of the Hexenal Transfer in the Liquid-Gas System

The rate of the hexenal transfer from the liquid to the gassy phase was studied to know the time necessary for the extraction. Hexenal (0.8 mM) was put in the 50 mL reactor containing glycine buffer 0.2 M pH 9 with total liquid volume of 15 mL. The flask crossed with nitrogen was connected at the exit to a flask put at −10°C and containing the hexane to facilitate the trap of the hexenal during the gas flowing.

Saturating Hexenal Concentration in the Gas Phase

The hexenal was removed from the liquid phase to the gas phase until reaching the saturation. This saturating concentration in the nitrogen has been determined experimentally in a hermetic flask of 1.8 mL. Different concentrations of hexenal have been diluted in 800 μL of glycine buffer and nitrogen passed to fill the flask. To determine the saturating concentration, an SPME fiber was completely placed in head-space during 20 min for the hexenal extraction.

Table 2 Eight run experimental design matrix.

Run	Identity element	X1	X2	X3	Interaction 12	Interaction 13	Interaction 23	Interaction 123	Response (%)
1	+	−	−	−	+	+	+	−	32
2	+	+	−	−	−	−	+	+	73
3	+	−	+	−	−	+	−	+	77
4	+	+	+	−	+	−	−	−	66
5	+	−	−	+	+	−	−	+	12
6	+	+	−	+	−	+	−	−	17
7	+	−	+	+	−	−	+	−	43
8	+	+	+	+	+	+	+	+	26
Contrast coefficients	43.25	2.25	9.75	−18.75	−9.25	−5.25	0.25	3.75	

Response (%) is yield of extraction (%) = (concentration of hexenal in the gassy phase/initial hexenal concentration in the liquid phase) × 100.

Sampling and Analysis of Volatiles

Hexenal concentration was measured by gas chromatography (GC). This compound was separated on (HP 5890 capillary column; 30 m×0.32 mm). Oven temperature was initially 70°C and increased after injection at 13°C min^{-1} up to 220°C. Helium carrier gas was used with a flow rate of 1 mL min^{-1}, and a flame ionization detector was set at 280°C. For determining the saturating hexenal concentration in the gassy phase (nitrogen), this compound was trapped on SPME fiber (60 μm polydimethylsiloxane/divinylbenzene) and was then thermally desorbed at 200°C for 2 min in the injector of GC.

Mathematical Model

Liquid–Gas Hexenal Transfer

It is important to designate two distinct hexenal concentrations in the partitioning reactor. The model is based on material balance around the reactor. Balance is made for hexenal concentration as a function of time. The hexenal transfer rate in the system is expressed as:

$$\frac{\mathrm{d\,Ad}}{\mathrm{d}\,t} = K_{\mathrm{L}}(\mathrm{Ad_s} - \mathrm{Ad}) \tag{1}$$

Where K_{L} is the gas phase mass transfer coefficient (m s^{-1}), $\mathrm{Ad_s}$ is the saturating hexenal concentration in gassy phase (mM), and Ad is the instantaneous concentration of hexenal in gas phase.

Mass Transfer Coefficient

$$K_{\mathrm{L}} = 2\sqrt{\frac{D}{\pi t}}\, K_{\mathrm{L}} = 2\sqrt{\frac{DU}{\pi L}} \tag{2}$$

Where U is the unidimensional average speed ($\mathrm{m}^2\ \mathrm{s}^{-1}$), L is the distance between the surface of liquid in the reactor and the outlet of gas (m): these two variables were determined experimentally, and D represents the coefficient of mass diffusivity of hexenal in the gassy phase ($\mathrm{m}^2\ \mathrm{s}^{-1}$).

Mass Diffusivity Coefficient

The mass diffusivity D_{AB} for binary mixtures is predictable within about 5% by kinetic theory. The corresponding formula of the system AB: CO–C_5H_{12} is given by Chapman-Enskog theory [11].

$$D_{\mathrm{AB}} = 0.0018583.\sqrt{T^3\left(\frac{1}{M_{\mathrm{A}}} + \frac{1}{M_{\mathrm{B}}}\right)}\frac{1}{p\sigma_{\mathrm{AB}}^2\Omega_{D(\mathrm{A,B})}} \tag{3}$$

M_{A} represents the molecular weight of CO, M_{B} is the molecular weight of C_5H_{12}, T is the temperature medium (K), P is the pressure (atm), σ_{AB} is the intermolecular force between CO and C_5H_{12} (A°) and $\Omega_{D\ (\mathrm{A,B})}$ is the collisional integral for diffusion of hexenal. $\Omega_{D\ (\mathrm{A,B})}$ is a function of the dimensionless temperature $KT/\varepsilon_{\mathrm{AB}}$.

The parameters σ_{AB} and ε_{AB} are those appearing in the Lennard-Jones potential between one molecule of A and one of B. σ_{AB} and ε_{AB} can be estimated from the following combining rules [12] :

$$\sigma_{AB} = 1/2\,(\sigma_A + \sigma_B) \tag{4}$$

$$\varepsilon_{AB} = \sqrt{\varepsilon_A \varepsilon_B} \tag{5}$$

The table of Lennard-Jones potential parameters [12] gives the following parameters:

$$\mathrm{CO}: \; M_A = 28 \;\; \sigma_A = 3.59\,A^\circ \;\; \varepsilon_A/K = 110\ \mathrm{K}$$
$$\mathrm{C_5H_{12}}: \; M_B = 72.15 \;\; \sigma_B = 5.81\,A^\circ \;\; \varepsilon_B/K = 327\ \mathrm{K}$$

The mixture parameters are then estimated from Eqs. 4 and 5:

$$\sigma_{AB} = 4.7\mathrm{A^o} \;\; \varepsilon_{AB}/k = \sqrt{110.(327-38)} \; = 178.29\,K \;\; \sin ce\ \varepsilon \mathrm{H_2}/K = 38$$

For a temperature of 313 K, the dimensionless temperature KT/ε_{AB}=1.75 K. The collision integral for diffusion of hexenal is determined from the table using Lennard-Jones potential for the prediction of transport properties of gases [13], $\Omega_{D(A,B)}$=1.128. Substitution of the preceding values in Eq. 3 gives: D_{AB}=9.1 10^{-6} $\mathrm{m^2\ s^{-1}}$

Then Eq. 2 gives: K_L=0.17 m $\mathrm{s^{-1}}$

System Simulation with Computer

Evolution of the component concentration was simulated with an iterative program (MATLAB 6.0). All the experimentally determined constants were introduced into the program.

Results and Discussion

The hexenal produced by lipoxygenase pathway has specific aromatic properties that can be used in various applications due to its green note. The expensive extraction of this compound has encouraged to search an easy way that allow to obtain a better efficiency of extraction with keeping a high purity of the molecule.

The principal objective of this study was the use of a liquid–gas biphasic system for the extraction of hexenal. This is a continuous agitated reactor in which hexenal continuously leaves the reactor by passing nitrogen as a carrier gas through the liquid phase (glycine buffer pH9) containing 0.8 mM of hexenal. The reactor temperature was maintained constant during the extraction, and the hexenal was driven by nitrogen to be trapped at −10°C. The first aspect of this research was the study of the variables influencing the transport of hexenal from the liquid to the gassy phase. Three important parameters needed to be improved. A quantitative study was performed with a 2^3 factorial design. The results presented by this work showed the great potential of this method to achieve optimization in a short number of experiments allowing the test of different parameters and the study of their interactions. Temperature is one of the key parameters in liquid/gas system because it has great influence on the hexenal partition between liquid and gas phases. Two temperatures were studied: the reactor temperature and the temperature of trap. The third parameter studied was the flow of nitrogen.

Experimental Design

A 2^3 experimental design was performed. It consists of eight runs that allowed the study of three parameters (see design matrix in Table 2). The values of each parameter at levels − and + are given in Table 1. The response was determined by the calculation of the yield of hexenal transfer. This yield was calculated for each treatment combination in the design according to the following formula:

$$\begin{aligned}&Y(\text{yield of the extraction})\\&\quad=(\text{concentration of hexenal in the gassy phase/initial hexenal concentration}\\&\qquad\text{in the liquid phase})\times 100.\end{aligned}$$

All experiments were conducted in duplicates, and the results were obtained as the average of the experiments under the same conditions (Table 2). According to the obtained contrast coefficients (Table 2), it maybe concluded that X1 (flow of nitrogen), X2 (temperature of the hexenal extraction), and X3 (temperature of trap) had an effect on the transport of hexenal. X2 and X3 appears to be the most influencing. However, the factor X1, when combined with the other factors, has an accentuated effect on the response. This is demonstrated by the study of interactions (Table 2), which shows that interaction 12, interaction 13, and the interaction of third order 123 has a significant contrast coefficients. The important yield of transfer (77%) was given for 40°C as reactor temperature and −10°C as temperature of the trap. These conditions (run 3) allowed a sixfold increase in the transfer of hexenal, when compared to run 5 in which the same flow of nitrogen was kept, and the other parameters were changed.

Transport Phenomena

The second aspect of this study was to analyze the transport phenomena such as mass transfer between the liquid and gas phases that causes a gradient in the concentration of hexenal. Thus, to provide adequate hexenal transfer from the liquid to the gas phases, the conditions improved previously must be carefully adjusted. The mass transfer coefficient calculated using Eq. 2 was used to determine the rate of hexenal transfer from the liquid to the gassy phase in the biphasic medium (glycine buffer/nitrogen). The observed mass transfer coefficient related to the gas phase corresponds to a value of 0.17 m s^{-1}.

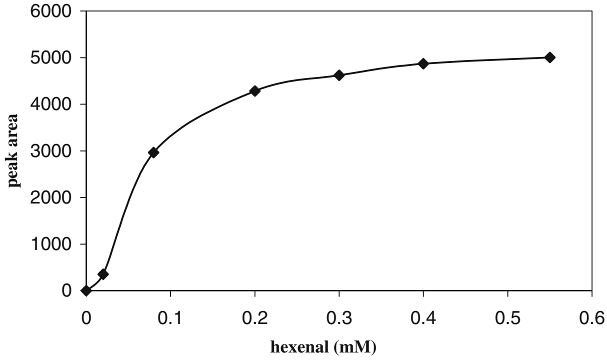

Fig. 1 Saturating hexenal concentration in the gassy phase (nitrogen). Hexenal in different concentrations was initially dissolved in aqueous phase which was in contact with a gas phase. The SPME fiber completely placed in head-space during 20 min allowed to measure the saturating hexenal concentration in nitrogen in each case

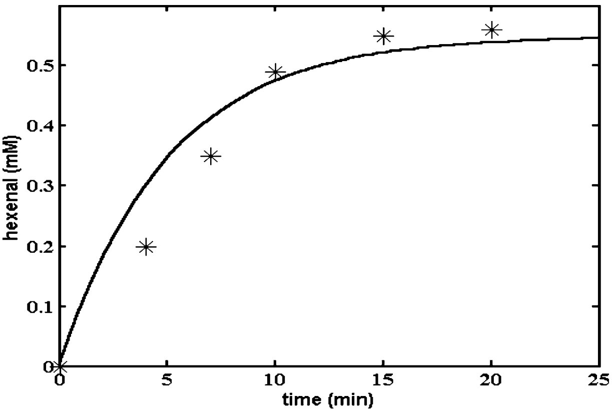

Fig. 2 Progress curve of hexenal transferred from the liquid to the gas phase (nitrogen) at reactor temperature of 40°C, trap temperature of −10°C, and nitrogen flow of 90 mL s^{-1}. The initial concentration of hexenal was 0.8 mM in the liquid phase. *Asterisks* Experimental results, *line* result of simulation

The hexenal transfer rate can be described using the Eq. 1: $\frac{\mathrm{d\,Ad}}{\mathrm{d}t} = K_{\mathrm{L}}(\mathrm{Ad_s} - \mathrm{Ad})$. Figure (1) shows the saturating hexenal concentration in the gas phase ($\mathrm{Ad_s}$). This concentration was determined experimentally in the nitrogen phase and was then analyzed with the SPME system. The data shows that this concentration was 0.5 mM. The progress curve of the hexenal transferred to the gas phase was determined in the biphasic system at the conditions previously improved with the experimental design (Fig. 2).

The coefficient of the mass transfer determined theoretically and the saturating hexenal concentration determined experimentally were used to simulate the transfer in the biphasic system using the Matlab program. The model simulates the evolution of reactants in the system as a function of time. The resulting simulated transfer progress curve and the experimental data are shown together in Fig. 2. The values given by the program are close to those determined experimentally. This shows a good correspondence between the experimentally determined and the modeled evolution of the hexenal concentration in the gas phase of the reactor. Modeled rate agreed with that determined experimentally. The aldehyde transport was stabilized at 20 min of the extraction, and the yield of hexenal transfer in the gas phase reached 77%.

Conclusion

The hexenal mass transfer in a liquid/gas biphasic system has been studied. The characteristics of the transport phenomena were investigated. The influence of the temperature and the flow of nitrogen were quantified. It was shown that temperature was the most important parameter affecting the transfer of aldehyde to the gas phase in the reactor.

With improved knowledge of this phenomenon, the actual mass transfer of hexenal in the two-phase system can be predicted with greater confidence and will be used in our modeling activities to predict the performance of the two-phase partitioning reactor. The extraction approach described here can serve later for the recovery of volatiles produced in enzymatic reactors using the enzymes of lipoxygenase pathway. The results found in this research demonstrated that the extraction of hexenal by passing nitrogen as a carrier gas through the liquid phase gives a maximum yield of transfer of 77%.

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