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ORIGINAL PAPER

The use of Macronet resins to recover γ-decalactone produced by *Rhodotorula aurantiaca* from the culture broth

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Abstract During the biotransformation of castor oil into γ-decalactone, *R. aurantiaca* produced both the lactone form and its precursor (4-hydroxydecanoic acid). After six days of culture, a maximum yield of γ-decalactone of 6.5 g/l was obtained. The parameters of γ-decalactone adsorption on three Macronet resins (MN-202, MN-102 and MN-100) were investigated in water. Adsorption isotherms of γ-decalactone for the three Macronet resins were linear. The trapping of γ-decalactone produced by *R. aurantiaca* on these resins was then carried out. γ-Decalactone was effectively retained by all the studied Macronet resins. The resin MN-202 trapped γ-decalactone more efficiently than MN-102 and MN-100. The percentages of γ-decalactone adsorbed on the resins MN-202, MN-102 and MN-100 were, respectively, 85, 75 and 81%, whereas around 70% of the adsorbed γ-decalactone was then desorbed. We propose an industrial process that uses Macronet resins to extract γ-decalactone from culture broth of *R. aurantiaca*.

Keywords γ-Decalactone · Extraction · Macronet resin · *Rhodotorula aurantiaca*

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Introduction

Lactones are aroma compounds that are widely used in the flavouring industry [1]. Among these lactones, γ-decalactone is a molecule with a fruity peach-like odour which, if produced by yeasts, can obtain a "natural" label. This aroma compound is produced by biotechnological methods using microorganisms, mainly yeast strains [2, 3]. The γ-decalactone concentrations reported in such processes vary from a few mg/l up to 11 g/l [4].

Product recovery is a difficult step in many bioprocesses, especially for flavour compounds because of their volatility and low solubility. The methods most commonly used to extract organic compounds from aqueous media involve solvent extraction, separation on specific membranes and adsorption on activated carbon [5–7]. In the last years, porous organic resins have replaced activated carbon as methods for extracting aroma compounds due to their hydrophobic nature and their high specific surface areas. Previous studies reported the adsorption of γ-decalactone onto activated carbon and hydrophobic resins (Porapak Q, Chromosorb 105 and SM4) by online extraction. However, while they limited the toxicity of the lactone towards the yeast, their presence in the bioconversion medium also decreased the production of γ-decalactone [8, 9].

We have previously investigated the effects of temperature, initial pH and castor oil concentration on the production of γ-decalactone by the psychrophilic yeast *Rhodotorula aurantiaca* A19. We found that *R. aurantiaca* produced the form *R*-γ-decalactone ((*R*)-(+)-4-hexylbutan-4-olide) (Fig. 1) [10]. Nevertheless, no data were published on the isolation and purification of γ-decalactone.

Davankov and Tsyurupa [11] disclosed their new series of hypercrosslinked networks in 1969. The polymers were originally described as being macroreticulated, offering

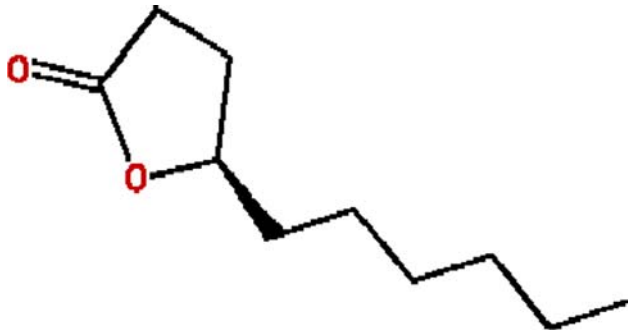

Fig. 1 *R*-γ-decalactone or (*R*)-(+)-4-hexylbutan-4-olide

characteristics different from those of other polymeric resins. An optimal series of Hypersol-Macronet™ sorbent resins for industrial application was developed by Purolite International Ltd. However, crosslinked polystyrene resins have turned out to be the most efficient adsorbents for recovering flavour compounds from aqueous solution [12, 13]. Valderrama et al. [14] described the sorption of six polycyclic hydrocarbons from an aqueous solution on the Macronet polymeric sorbent MN-200. In the present work, the use of Macronet resins in culture broth to extract the lactone produced by *R. aurantiaca* was evaluated. To our knowledge, this is the first report discussing the utilization of Macronet resins for extracting lactones from production media.

Materials and methods

Yeast cultures and media

Rhodotorula aurantiaca is a psychrophilic strain previously isolated near the Antarctic Station Dumont d'Urville and deposited at the Mycotheque of the University of Louvain-la-neuve (MUCL), Belgium, under registration No. 40267. The yeast was grown in glucose medium as previously described [10]. This yeast was used in the present study for γ-decalactone production. The biotransformation medium was composed of 6 g/l of peptone casein, 3 g/l of yeast extract and 20 g/l of castor oil.

Biotransformation of castor oil into γ-decalactone

A 20-l fermentor was used, with 12 l of biotransformation medium. All fermentor trials were conducted with aeration (1.5 vvm) and at constant temperature (14°C), pH (6.8) and agitation (350 rpm), by online control using Freelance 2000 software. The pH was controlled by the automatic addition of 6 M NaOH and 6 M H_3PO_4 solutions. The fermentation was stopped when the γ-decalactone concentration reached its maximum and cells entered the stationary phase.

Table 1 Characteristics of the synthetic Macronet resins

Commercial name	Functional groups	Specific gravity (g/ml)	Specific area (m^2/g)
Macronet MN-100	Tertiary amine	1.09	900
Macronet MN-102	Tertiary amine	1.07	700
Macronet MN-202	None	1.04	700

To increase the lactonization of 4-hydroxydecanoic acid into γ-decalactone, the fermentor content was acidified at pH 2.0 using concentrated sulfuric acid and heated at 95°C for 30 min [15]. After cooling and centrifugation, γ-decalactone was then extracted from the culture broth by adsorption on hydrophobic Macronet resins (MN-202, MN-102 and MN-100).

Resins

Three Macronet resins were tested: MN-202, MN-102 and MN-100. All three resins are polystyrene crosslinked with divinylbenzene. The resins were purchased from Purolite International (Paris, France). Characteristics of the resins are presented in Table 1. The resins were cleaned for 24 h with ethanol. The washed resins were placed at 80°C for 6 h in order to eliminate the remaining ethanol.

Adsorption of γ-decalactone on Macronet resins

The adsorption of γ-decalactone on the different Macronet resins was achieved at 25°C. To investigate the adsorption kinetics of γ-decalactone on each resin, an aqueous solution of γ-decalactone at 0.6 g/l was prepared. The resins were added to the solution at a concentration of 30 g/l and stirred at 400 rpm. The influence of the ratio resin/γ-decalactone and of the regeneration of resins on the extraction of γ-decalactone was studied.

Recovery and quantification of the adsorbed γ-decalactone from the resins

Adopting the procedure of Matsukura et al. [16], the resin was removed from the culture broth, washed several times with distilled water, then placed over a paper filter and air-dried at room temperature for 1 h. The organic products were extracted with three volumes of ethanol at a ratio of 4 ml/g resin. For quantification, a sample of the ethanolic fraction was collected and dried overnight over anhydrous sodium sulfate, and γ-valerolactone was added as an internal standard. Direct injection of the ethanolic extract was then performed by GC.

In order to determine the purity of γ-decalactone, 50 mg of concentrated γ-decalactone produced by *R. aurantiaca*

was dissolved in 100 ml of diethyl ether and compared by GC analysis to the commercial γ-decalactone (Sigma–Aldrich).

GC analyses were performed using a 5890 series II gas chromatograph from Hewlett-Packard (Palo Alto, CA, USA) equipped with a flame ionisation detector and an All-tech AT AQUAWAX column (30 m × 0.25 mm ID, film thickness 0.25 μm). The oven temperature was held at 40°C for 2 min, raised to 250°C at a rate of 10°C/min, then fixed at 250°C for 20 min. The injector and detector temperatures were 200 and 250°C, respectively. The carrier gas, helium, was adjusted to a linear velocity of 1 ml/min and 0.5 bars. One-microlitre samples were injected into the GC apparatus.

Results and discussion

Production of γ-decalactone and 4-hydroxydecanoic acid by *R. aurantiaca* in a 20-l fermentor

During the biotransformation of castor oil (a source of ricinoleic acid), *R. aurantiaca* produced four lactones: γ-octalactone, γ-nonalactone, γ-decalactone and γ-undecalactone. Among these lactones, γ-decalactone was the most abundant [10]. In biotransformation medium, *R. aurantiaca* produced both γ-decalactone and its precursor 4-hydroxydecanoic acid. Maximum γ-decalactone and 4-hydroxydecanoic acid concentrations (2 and 4.5 g/l, respectively) were obtained at a medium pH of 6.8 (data not shown).

The influence of pH on the extraction of γ-decalactone was investigated in our study. Table 2 shows the recovery of γ-decalactone with or without acidification just before the extraction. The results obtained show that at a medium pH of 6.8, some of the γ-decalactone is not extractable using diethyl ether as solvent. Acidification of the culture to pH 2.0 prior to the extraction increased the γ-decalactone concentration 2- and 2.6-fold compared to media at pH 3.5 and 6.8, respectively. This is due to the spontaneous cyclization of 4-hydroxydecanoic acid into the neutral lactone form. However, this reaction is not total, and some of the acid is not transformed into γ-decalactone. Similar results were obtained by Dufossé et al. [17] and Feron et al. [18], who found that acidification of the cultures to pH 2.0 enhanced the recovery of γ-decalactone produced by *Sporidiobolus ruinenii*.

The lactonization of 4-hydroxydecanoic acid into γ-decalactone was enhanced by heating the culture broth at 95°C for 30 min. After six days of culture, the concentration of γ-decalactone obtained by *R. aurantiaca* was around 6.5 g/l (Table 2). The results obtained using the yeast *R. aurantiaca* in the fermentor was on the order of the concentrations usually described in patents. Consequently, the bioproduction of γ-decalactone with *R. aurantiaca* includes a two-step procedure, i.e. the biotransformation of castor oil into 4-hydroxydecanoic acid and the lactonization of this acid (after acidification and heating).

Table 2 Influence of broth pH and heating on the recovery of γ-decalactone produced by *R. aurantiaca* after six days of culture

pH of medium	γ-Decalactone (g/l)	SD
6.8	2.1	±0.3
3.5	4.2	±0.4
2.0	5.5	±0.6
pH 2.0 and heating at 95°C for 30 min	6.5	±0.7

γ-Decalactone was then extracted from the culture broth by adsorption on hydrophobic Macronet resins (MN-202, MN-102 and MN-100). Some aspects of the adsorption of γ-decalactone onto these three resins from aqueous media will be discussed below, before we describe their application in the production medium.

Adsorption kinetics

The adsorption kinetics of γ-decalactone on the three Macronet resins were studied in water with 0.6 g/l of γ-decalactone and 30 g/l of each resin. The decrease in the level of γ-decalactone in the aqueous solution was followed by analysis every 10 min until the remaining quantity of γ-decalactone reached equilibrium. The quantity adsorbed corresponded to the difference between the initial concentration and the final concentration (after the adsorption).

γ-Decalactone was adsorbed very quickly onto the resins MN-202 and MN-100. After 20 min, 21% of the γ-decalactone initially present in the aqueous solution remained in the case of MN-202, and 29% in the case of MN-100. The kinetics of adsorption onto the resin MN-102 were different from those onto the other resins, and 65% of the γ-decalactone was still present in the aqueous solution after 20 min. After about 40 min of contact, equilibrium was reached for all of the resins (Fig. 2). The initial adsorption rate of γ-decalactone on the three resins was estimated on the basis of the first 10 min. Table 3 shows that the initial adsorption rate of γ-decalactone onto the resin MN-202 was higher than those for the other resins. Hydrophobic interactions, chemical structure, and high specific surface area are the main driving forces for the adsorption of γ-decalactone on Macronet resins in aqueous media, because this aroma compound has a low solubility in water [19]. Thus, the capacity of the resin MN-100 to adsorb γ-decalactone from aqueous media is higher than that of the resin MN-102 because of its higher specific surface area (900 and 700 m^2/g, respectively), whereas the difference in adsorption between the resins MN-202 and MN-102 can be attributed to their

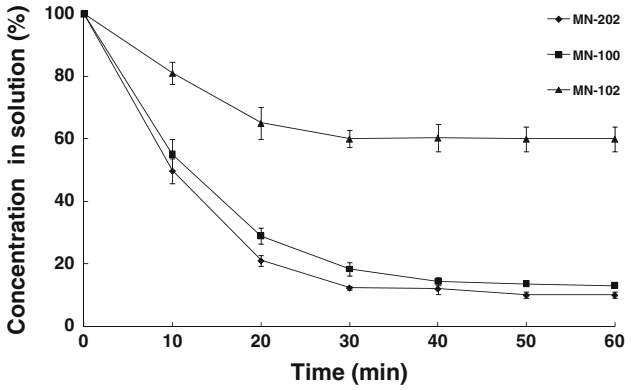

Fig. 2 Adsorption kinetics of γ-decalactone on Macronet resins (MN-202, MN-100 and MN-102) in aqueous media at 25°C. The γ-decalactone and resin concentration used were 0.6 and 30 g/l, respectively

Table 3 Initial adsorption rate of γ-decalactone on the Macronet resins (MN-202, MN-100 and MN-102) after 10 min

Resin (30 g/l)	MN-100	MN-102	MN-202
Initial concentration of γ-decalactone (g/l)	0.6	0.6	0.6
Adsorbed quantity of γ-decalactone (g/l) after 10 min	0.25	0.1	0.3
Initial adsorption rate	0.83×10^{-3}	0.33×10^{-3}	1×10^{-3}

The rate is expressed in grams of γ-decalactone per gram of resin per minute

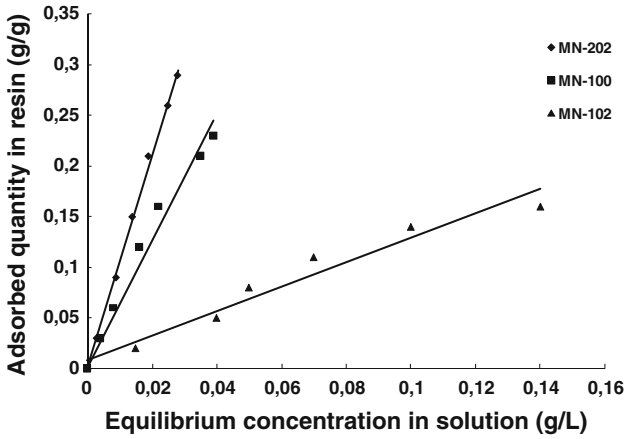

Fig. 3 Adsorption isotherms of γ-decalactone on the Macronet resins (MN-202, MN-100 and MN-102) in aqueous media at 25°C. Equilibrium γ-decalactone concentrations were measured in the range 0.1–0.6 g/l using 30 g/l of each resin

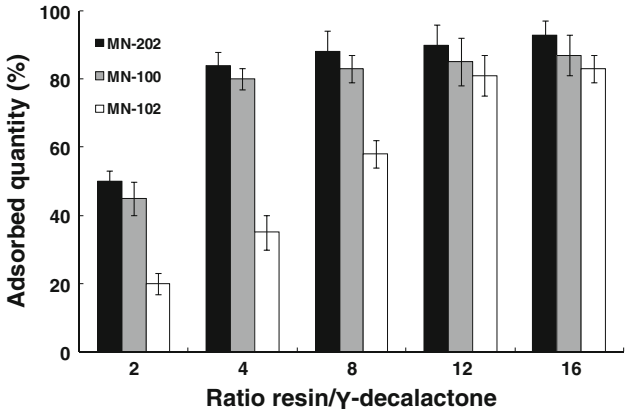

Fig. 4 Percentages of adsorbed γ-decalactone on the Macronet resins (MN-202, MN-100 and MN-102) as a function of the ratio resin/γ-decalactone

chemical structures, since they have the same specific surface area (700 m^2/g).

Adsorption isotherms and the ratio resin/γ-decalactone

Determining the adsorption isotherm is an important task in relation to characterizing the adsorption of the aroma compound onto the resins. In our study, equilibrium γ-decalactone concentrations were measured in the range 0.1–0.6 g/l. The resins were added to the solution at a concentration of 30 g/l. Our results show that the isotherms of γ-decalactone for the three Macronet resins (MN-202, MN-102 and MN-100) were linear in this range of concentrations (Fig. 3). This linearity can be attributed to the low solubility of γ-decalactone in water (0.6 g/l). These results are in agreement with the findings of Souchon et al. [19], who found that the isotherms of γ-decalactone for activated carbon, Amberlite XAD-4, Chromosorb 105 and Porapak Q were also linear. However, the adsorption behaviour of the aroma compound can be related to the solubility of the compound.

In order to determine the quantity of resin to use, the influence of the ratio resin/γ-decalactone on the extraction of γ-decalactone by adsorption on the three Macronet resins was investigated. The ratio expresses the number of grams of resin employed per gram of product. Figure 4 shows that the ratio resin/γ-decalactone strongly influenced the adsorption of γ-decalactone onto the studied resins, especially on MN-102. For a ratio of 2, the percentage of adsorption onto the resins was between 20 and 50%, whereas for a ratio of 4, 84% of the product initially present in the water was fixed onto the resin MN-202 and 80% was adsorbed onto MN-100. MN-102 traps γ-decalactone less efficiently than MN-202 and MN-100. The adsorption of γ-decalactone onto MN-102 rose as the ratio MN-102/γ-decalactone increased. However, for ratios of 12 and 16, there was no significant difference in the adsorption of the lactone onto the three resins. Thus, the ratios selected to perform the next steps were 4 for the resins MN-202 and MN-100 and 12 for the resin MN-102.

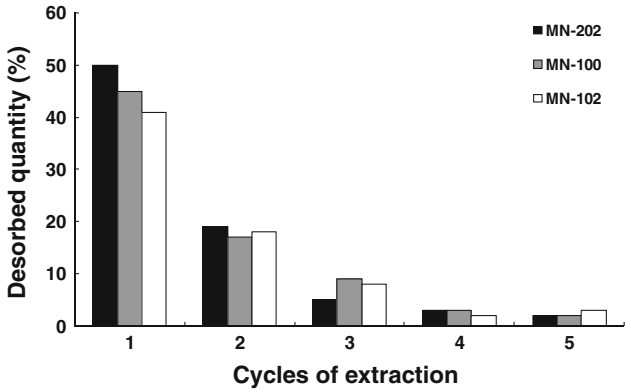

Fig. 5 Desorption of the γ-decalactone fixed onto the Macronet resins (MN-202, MN-100 and MN-102) using five volumes of ethanol

Desorption of γ-decalactone

Efficient stripping of γ-decalactone from the resins was achieved using ethanol. The recovery of the amount adsorbed onto the total mass of each resin was performed with three volumes of ethanol at a ratio of 4 ml/g resin. The resins used showed good capacities for desorption. Figure 5 show the percentages of γ-decalactone desorbed from the Macronet resins by ethanol after five cycles of extraction. The amounts desorbed were 75% for MN-202, 71% for MN-100, and 67% for MN-102.

Regeneration of the resin

The regeneration of the Macronet resins (MN-202, MN-102 and MN-100) was investigated by a batchwise method where ethanol was used to remove the adsorbed γ-decalactone (five cycles of extraction). We carried out a regeneration study of these resins by analyzing six cycles of adsorption at 25°C. The results obtained demonstrated that all of the resins can be reused at least six times. In all cases, and even after six cycles, the percentage adsorption was higher than 80%.

Adsorption of γ-decalactone produced by *R. aurantiaca* on Macronet resins

Studying the adsorption of γ-decalactone onto Macronet resins in water permitted us to determine the experimental conditions (adsorption time, amount of resin required to extract γ-decalactone, and volume of ethanol needed to desorb it) required for the recovery of γ-decalactone produced with *R. aurantiaca* from culture broth.

The adsorption of γ-decalactone produced by *R. aurantiaca* onto the resins was investigated. γ-Decalactone was strongly adsorbed onto all Macronet resins (from 75% for MN-102 to 85% for MN-202). Among these resins, MN-202 was the most efficient adsorbent for γ-decalactone. The percentage desorption was around 70% of the adsorbed γ-decalactone, with a purity of 80%. The level of adsorption of γ-decalactone from the culture broth of *R. aurantiaca* onto Macronet resins was lower than that observed in water. This difference can be attributed to the presence of castor oil and biomass in the medium, which adsorbed some of the compound. The castor oil used as substrate acts not only as a biotransformation precursor but also as a lactone extractant. However, this oily phase is progressively reduced during the process.

Conclusion

The adsorption of γ-decalactone onto Macronet resins is a suitable method for extracting γ-decalactone from culture broth. The usefulness of Macronet resins for industrial applications results from a number of advantages: the studied resins are not chemically reactive with the aroma compounds; they are easily regenerated; they are not expensive; and they can easily be adapted to an industrial scale. Moreover, their elution requires only small volumes of ethanol, which can also be recycled.

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