Research Article

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Production of γ-decalactone by *Yarrowia lipolytica*: insights into experimental conditions and operating mode optimization

Adelaide Braga and Isabel Belo*

Abstract

BACKGROUND: γ-Decalactone production from ricinoleic acid biotransformation derived from the triglycerides in castor oil by *Yarrowia lipolytica*, has been widely described in literature in studies concerning lipidic metabolism that leads to lactones production, interactions of cells with the lipid substrate, toxicity of produced metabolites, selection of over-producing mutants and selection of environmental conditions.

RESULTS: In order to improve technological aspects of γ-decalactone production, oxygen transfer rate (OTR), cell density and oil concentration effects were investigated, in batch and step-wise fed-batch cultures of *Yarrowia lipolytica* W29. The best γ-decalactone concentration of 5.4 ± 0.5 g L^{-1} was obtained for batch cultures with 60 g L^{-1} of cells and substrate concentration.

CONCLUSION: The direct influence of aeration and agitation rates, thus of OTR, on production of γ-decalactone has been demonstrated. γ-Decalactone productivity of 215 ± 19 mg L^{-1} h^{-1} was obtained with 60 g L^{-1} of cells and castor oil concentration in batch and step-wise fed-batch cultures of *Yarrowia lipolytica*. The results obtained suggest that these two strategies are good alternatives for industrial production processes.

Keywords: castor oil; biotransformation; *Yarrowia lipolytica*; γ-decalactone; batch and step-wise fed-batch cultures

INTRODUCTION

The market for natural products is booming as a result of consumer demand.[1] The biotechnological production of γ-decalactone (a peach-like aroma compound) is of particular interest since it is generally encountered in very low concentrations in plant or animal tissues and is largely applied at industrial level in food and beverages, soaps, cosmetics, chemicals, pharmaceuticals, detergents and toiletries.[2] For this purpose, it can be obtained from the biotransformation of ricinoleic acid catalyzed by *Yarrowia lipolytica*, a yeast with GRAS status.[3] This yeast species is able to grow on hydrophobic substrates such as oils, n-alkanes, fats and fatty acids, for which it has specific metabolic pathways. The main metabolic pathway of fatty acids is through β-oxidation peroxisomal.[4] Fatty acids in the form of esters of coenzymeA are degraded in a sequence of four steps resulting in a two-carbon chain-shortening. The pathway from ricinoleyl-CoA to γ-decalactone involves four β-oxidation cycles yielding 4-hydroxy-decanoyl-CoA, which is then, cyclized to γ-decalactone (Fig.1(a)). β-oxidation of acyl-CoA shorter than C_{10} leads to the production of other aroma compounds different from γ-decalactone, such as 3-hydroxy-γ−decalactone.[5] The first step of one β-oxidation cycle is catalyzed by acyl-CoA oxidase and then by three multifunctional enzymes: 2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase[6] (Fig. 1(b)). The activities of these enzymes can be influenced by oxygen, since it is necessary for the regeneration of the cofactors FAD and, more indirectly, NAD^+ and therefore influences the production of γ-decalactone.[7]

As an intervening factor in the metabolic pathway involved in this biotransformation, oxygen plays an important role in the reactions of production and consumption of the aroma. For this reason it is imperative to define the most appropriate conditions of oxygenation for lactones industrial production. There are several reports in the literature describing the effect of operating conditions on the production of lactones carried out by *Y. lipolytica*; however, some discrepancies are apparent, especially concerning the effect of oxygen.[8−10] Previous studies[9] demonstrated the influence of operating conditions on oxygen mass transfer and on γ-decalactone production from methyl ricinoleate in a stirred tank bioreactor. Gomes *et al.*[9] obtained a maximum aroma concentration of 141 mg L^{-1} at agitation and aeration rates of 400 rpm and 0.6 vvm, respectively.

Besides the effect on fatty acids metabolism, oxygen is particularly important to *Y. lipolytica* as an aerobic microorganism, and due to its low solubility in aqueous solutions, a continuous oxygen transfer rate from the gas phase to the culture medium is required. In general, the dissolved oxygen concentration variation in batch

* *Correspondence to: I. Belo, IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. E-mail: ibelo@deb.uminho.pt*

IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal

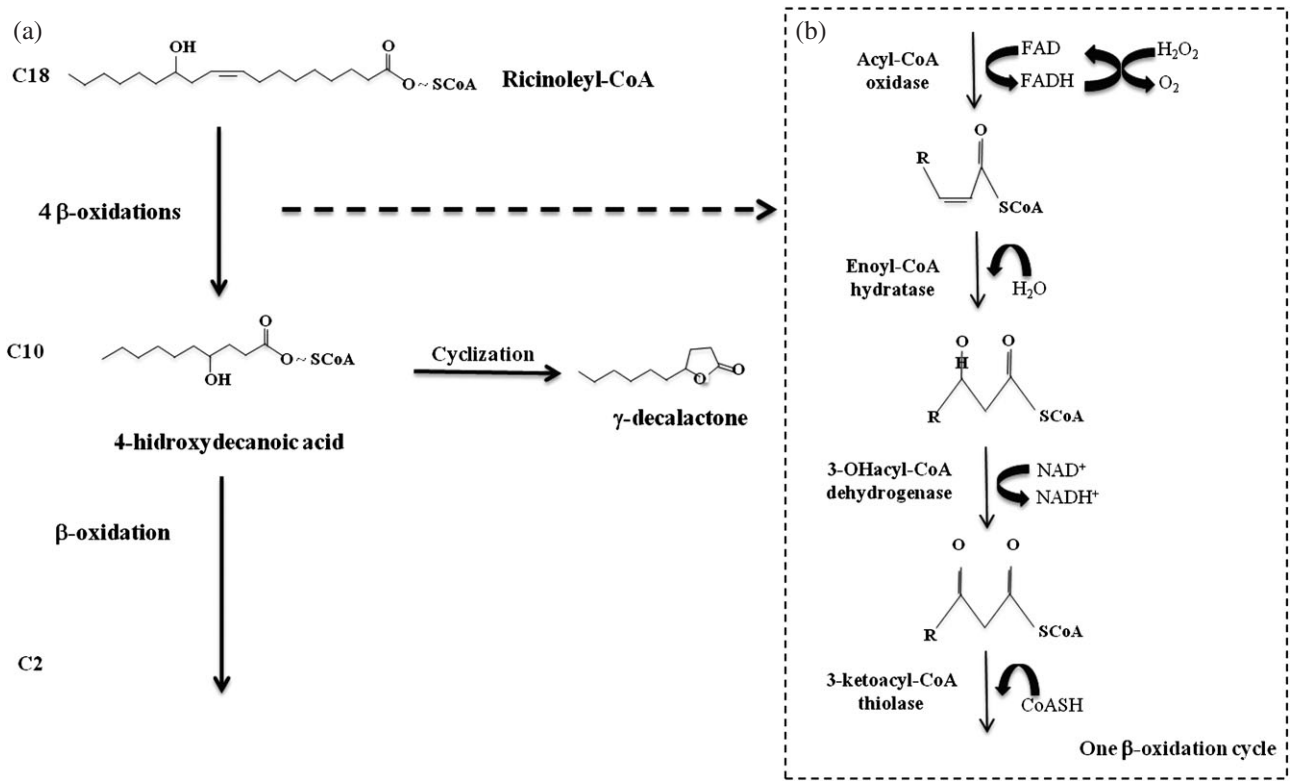

Figure 1. The pathway from ricinoleyl-CoA to γ-decalactone (a) and enzymes involved in the yeast peroxisomal β-oxidation (b).[6,37]

bioreactors is described by the following mass balance equation:

$$dO/dt = OTR - OUR \quad (1)$$

where O represents the dissolved oxygen concentration within the medium, t the time, OUR the oxygen uptake rate and OTR the oxygen transfer rate.

The oxygen uptake rate in a culture depends on cell density (X) and on the specific oxygen uptake rate (q_{O2}), as shown in Equation 2:

$$OUR = q_{O2}X \quad (2)$$

Also, the oxygen transfer rate in a system is a function of the volumetric mass transfer coefficient, k_La, and the driving force resulting from the difference between the oxygen saturation (O^S) and the dissolved oxygen concentration in the liquid phase:[11,12]

$$OTR = k_La\ (O^S - O) \quad (3)$$

OTR and k_La determination are essential for the characterization of bioreactors in aerobic processes. Through these parameters it is possible to determine the optimum aeration and agitation rates and to assess the effect of each on oxygen transfer rate.[13]

Besides environmental conditions, the effect of medium composition on γ-decalactone production has been studied. Different substrates were described for aroma production such as castor oil and their hydrolysates, fatty acids or esters of these compounds,[14] including methyl ricinoleate, and sugars such as glucose and fructose.[15] The influence of castor oil on the yields of γ-decalactone was reported by Alchihab *et al.*[16] in flask experiments. The authors showed that γ-decalactone concentration obtained was not directly proportional to the concentration of castor oil in the medium. The highest γ-decalactone production (5.5 g L^{-1}) was recorded with 20 g L^{-1} of castor oil with a decrease for higher castor oil contents. However, Dufossé *et al.*[17] showed that γ-decalactone concentration obtained in the culture of *Sporidiobolus ruinenii* increased almost proportionally with ricinoleic acid methyl ester concentration between 0 and 160 g L^{-1}. Besides substrate concentration, cell density in the culture may also interfere with aroma production. An *et al.*[18] conducted some experiments at different cell concentrations (0–50 g L^{-1}) and analyzed its impact on γ-dodecalactone production by permeabilized *Waltomyces lipofer* cells. They observed that at concentrations higher than 30 g L^{-1} of permeabilized cells, γ-dodecalactone production reached a plateau, suggesting the existence of an optimal cell concentration of 30 g L^{-1} for this strain and process.

Fed-batch operation allows higher cell density than batch mode and is often applied to obtain high yields and productivities, by controlling the nutrient feeding.[19] With this approach it is possible to supply more substrate to the cells, simultaneously preventing eventual toxic effects. Lee *et al.*[20] used step-wise fed-batch cultures in γ-decalactone production by *Sporobolomyces odorus*, where cell growth and γ-decalactone production were improved by the addition of castor oil hydrolysate once per day for 3 days. They found that the maximum productivity of γ-decalactone (1.23 mg L^{-1} h^{-1}) increased with the number of castor oil hydrolysate feedings. Gomes *et al.*[21] obtained a γ-decalactone productivity of 43 mg L^{1} h^{-1} for a step-wise fed-batch operation applied to *Y. lipolytica* cultures, when 30 g L^{-1} methyl ricinoleate was fed twice to the bioreactor.

Nevertheless, most processes described in the literature reported low aroma concentration rarely reaching more than 11 g L^{-1} in the fermentation broth.[22] For example, using

a uracil auxotrophic mutant *Y. lipolytica* strain (Leu⁻ Ura⁻), Nicaud *et al.*[23] obtained high production of γ-decalactone from methyl ricinoleate at the end of the growth phase, achieving an aroma productivity of 127 mg L^{-1}·h^{-1}. In a production process established by Haarmann and Reimer GmbH (H&R) in Germany, a productivity of 169 mg·L^{-1}·h^{-1} was reported, using a non-genetically modified strain and castor oil as raw substrate.[24]

The main aim of this present study was to analyze different strategies to improve γ-decalactone production by *Y. lipolytica* W29 on a medium containing castor oil, as ricinoleic acid source, focusing on experimental conditions: agitation and aeration rates that influence oxygen transfer rate, cell and substrate concentration and also different culture modes of operation (batch and step-wise fed-batch).

EXPERIMENTAL PROCEDURES

Microorganism, media and culture conditions

Yarrowia lipolytica W29 (ATCC20460) was cultured for 48 h on YPDA medium (agar 30 g·L^{-1}, glucose 20 g·L^{-1}, peptone 20 g·L^{-1}, yeast extract 10 g·L^{-1}) at 27 °C to inoculate (cell density of 0.5 g·L^{-1}) 500 mL baffled Erlenmeyer flasks containing 200 mL of glucose medium (YPD medium: glucose 20 g·L^{-1}, peptone 20 g·L^{-1}, yeast extract 10 g·L^{-1}). Flasks were incubated at 140 rpm, 27 °C for 19 h. Cells from this pre-inoculum were harvested by centrifugation (6000 rpm, 10 min) and used to inoculate 1.7 L of YPD medium in the bioreactor, to give an initial cell concentration of 0.5 g·L^{-1}. After the cell growth phase, biotransformation takes place. The components of the biotransformation medium were added to the YPD medium containing the cells, in order to start the biotransformation phase. The composition of the biotransformation medium was 6.7 g·L^{-1} YNB (yeast nitrogen base) with amino acids, 2.5 g·L^{-1} NH_4Cl, 30 g·L^{-1} or 60 g·L^{-1} castor oil (CO) and 3 g·L^{-1} or 6 g·L^{-1} Tween 80, respectively.

Bioreactor

Biotransformations were carried out in a 3.7 L operating volume bioreactor (RALF PLUS SOLO, Bioengineering, Switzerland). Air was supplied with a sparger located at the base of the agitator, with a flow-rate of 1.7 L·min^{-1} and 5.1 L·min^{-1} and stirring rates of 400 rpm, 500 rpm and 650 rpm.

For k_La determination the dynamic gassing-out technique was used. In the presence of active cells and in the absence of aeration, Taguchi and Humphrey[25] used the respiratory activity of microorganisms to remove oxygen from the medium. This technique has been performed during fermentation. The procedure involves two steps: one to stop aeration and another for resumption of aeration in the operating conditions. Thus, in the first step, monitoring the decrease of dissolved oxygen concentration will allow determination of the specific oxygen uptake rate (OUR).

$$dO/dt = -OUR \quad (4)$$

Aeration is resumed before reaching the critical dissolved oxygen concentration value.[11] After the resumption of aeration (second step), the oxygen mass balance in the liquid phase is expressed by Equation 5:

$$dO/dt = k_La\ (O^s - O) - OUR \quad (5)$$

Considering the pseudo-steady state immediately before the determination, OUR can be replaced by Equation 6:

$$k_La\ (O^s - O_i) = OUR \quad (6)$$

where O_i is the dissolved oxygen concentration at the beginning of the determination. By integration of this last equation, we obtain:

$$\ln\left(\frac{O_i - O}{O_i - O_o}\right) = -k_La\ (t - t_0) \quad (7)$$

where O_0 and t_0 are, respectively, the dissolved oxygen concentration and the time when aeration is resumed.

The graphical representation of the term on the left side of Equation 7, as a function of time, gives a line whose slope is the k_La value.

The medium pH of 6.0 was controlled by addition of 2 N KOH or 21% (v/v) orthophosphoric acid, through Peripex peristaltic pumps (Bioengineering, Switzerland). Two different cell concentrations of 30 g·L^{-1} and 60 g·L^{-1} (dry mass) were used for the biotransformation. Cellular growth occurred at 27 °C, 500 rpm and 3 L·min^{-1} of aeration rate for 19 h until a final cell density of 30 g·L^{-1} and total glucose consumption. The cell concentration of 30 g·L^{-1} was obtained in a batch culture in 1.7 L of YPD medium for 21 h, while the 60 g·L^{-1} cell density was reached by fed-batch growth with constant flow rate (0.3 mL·min^{-1}) of 5-fold concentrated YPD medium (0.4 L), after the batch phase (in 1.3 L of YPD), for 24 h. After the cell growth phase, for each cellular density, biotransformations were performed with two different castor oil concentrations, 30 g·L^{-1} as in previous work[10,21] and double this concentration (60 g·L^{-1}).

The influence of substrate was also analyzed in a step-wise fed-batch strategy, based on two additions of castor oil (30 g·L^{-1} and 60 g·L^{-1}) for the two cell concentrations studied.

Sampling and analytical methods

Samples were collected over time for analysis of cell concentration and for γ-decalactone quantification. Cell concentration was estimated using a Neubauer-improved counting chamber.[26] γ-Decalactone was extracted from 2 mL samples with 2 mL diethyl ether and the organic phase was analyzed by gas chromatography (GC) as previously described by Braga *et al.*[27]

RESULTS AND DISCUSSION

Influence of oxygen in γ-decalactone production

In microbial cultivation processes, productivity is often limited by the transport of a substrate, in which oxygen is one example. Therefore, in order to evaluate the impact of medium oxygenation on the biotransformation process, experiments were carried out varying the oxygen transfer rate from air to the culture medium, through manipulation of the agitation and aeration rates.

As expected, an increase in agitation from 400 rpm to 500 rpm and aeration rate from 1.7 L·min^{-1} to 5.1 L·min^{-1} resulted in a 2-fold increase of the k_La. Moreover, k_La improvement was significantly influenced by agitation, since an increase from 500 rpm to 650 rpm at constant air flow rate also led to a 1.6-fold increase in k_La. This behavior is in agreement with the results of Amaral *et al.*,[28] Gomes *et al.*,[9] Gómez-Diaz and Navaza[29] which showed that k_La depends more strongly on agitation than on aeration rates.

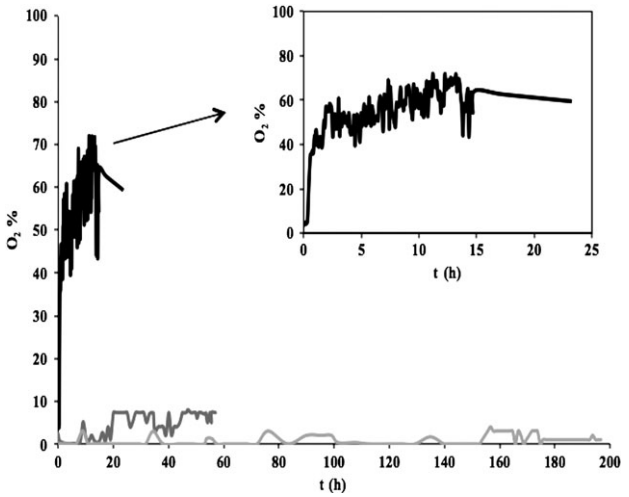

Figure 2. Time course of dissolved oxygen concentration during the biotransformation phases in the bioreactor under different agitation and aeration rates: (—) 650 rpm and 5.1 L·min^{-1}; (—) 500 rpm and 5.1 L·min^{-1} and (—) 400 rpm and 1.7 L·min^{-1}. Experiments performed with 30 g L^{-1} of cell and substrate concentration.

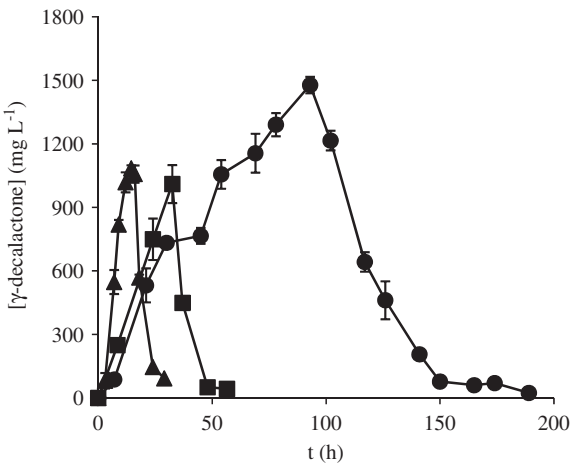

Figure 3. Accumulation of γ-decalactone in the biotransformation medium under different operating conditions in the bioreactor: (■) 400 rpm and 1.7 L·min^{-1}; (▲) 500 rpm and 5.1 L·min^{-1}; (●) 650 rpm and 5.1 L·min^{-1}. Experiments performed with 30 g L^{-1} of cell and substrate concentration. Data are presented as mean and standard deviation of two independent experiments.

Table 1. Volumetric mass transfer coefficient (k_La), Specific oxygen uptake rate (q_{O2}) and maximum γ-decalactone productivity, obtained with 30 g L^{-1} of cell and substrate concentration, under different operating conditions. Data are presented as the mean and standard deviation of two independent experiments

Experimental conditions (Agitation and aeration rate)	k_La (h^{-1})	q_{O2} ($mg{\cdot}L^{-1}{\cdot}h^{-1}$)	Productivity ($mg{\cdot}L^{-1}{\cdot}h^{-1}$)
400 rpm 1.7 L min^{-1}	39 ± 3	3. 1 ± 0.2	16 ± 6
500 rpm 5.1 L min^{-1}	69 ± 3	11.2 ± 0.5	31 ± 8
650 rpm 5.1 L min^{-1}	113 ± 5	14.3 ± 0.7	75 ± 10

By analyzing the specific oxygen uptake rate (q_{O2}) values it is possible to observe that q_{O2} increases with k_La. These confirm the results in Fig. 2 since oxygen limitation is observed for the lowest OTR conditions tested, suggesting that the maximum q_{O2} may be attained by further improving k_La.

It is also possible to observe that low agitation and aeration rates led to a complete depletion of dissolved oxygen. In contrast, when using high agitation and aeration rates a stable dissolved oxygen concentration, between 40 and 60%, was observed throughout the experiment.

From Table 1 it is clearly observed that k_La has a positive influence on q_{O2} and on aroma productivity. This increase in γ-decalactone productivity is a consequence of the increase in production rate and not on the maximum aroma concentration obtained (Fig. 3).

In fact, a higher aroma concentration was obtained at low oxygenation rates, but the time needed to reach the peak of γ-decalactone production was reduced with higher OTR, resulting in higher productivities when compared with that obtained using low aeration (Table 1). In previous studies by Aguedo *et al.*[30] different agitation and aeration rates were tested in the production of γ-decalactone; however, a different bioreactor and also a different substrate (methyl ricinoleate) were used. Their results presented very low productivities (1 – 13 $mg{\cdot}L^{-1}{\cdot}h^{-1}$) when compared with those achieved in this work. The low oxygen concentration does not only affect acyl-CoA oxidase activity, but selectively switches to other pathways in the β-oxidation cycle and affects 3-hydroxyacyl-CoA dehydrogenase through the regeneration of NAD (respiration-dependent), resulting in higher yields of other compounds such as 3-hydroxy-γ – decalactone.[31]

For all the conditions studied, the kinetic profile of γ-decalactone production (Fig. 3) is typical for this strain,[8,9] increasing to a maximum followed by a decrease in aroma production until complete disappearance of the compound (Fig. 3). The γ-decalactone disappearance is due to the ability of yeasts to consume the aroma as a carbon source.[30] The maximum aroma concentration obtained was around 1.5 ± 0.4 $g{\cdot}L^{-1}$, when the agitation and aeration rates used were 400 rpm and 1.7 L·min^{-1}, the lowest oxygenation conditions tested.

This result is in agreement with the results obtained by Aguedo *et al.*[8] and García *et al.*[32] Both of these works concluded that low oxygen concentrations in the medium induce control of the β-oxidation pathway by acyl-CoA oxidase and therefore, an accumulation of γ-decalactone occurs.

Influence of cell and substrate concentration

The influence of different castor oil concentrations on γ-decalactone production was investigated. Figure 4 indicates a maximum γ-decalactone concentration of 5.4 ± 0.5 $g{\cdot}L^{-1}$, obtained at 25 h, with 60 $g{\cdot}L^{-1}$ of castor oil and a cell density of 60 $g{\cdot}L^{-1}$.

After the achievement of maximum lactone concentration with 30 $g{\cdot}L^{-1}$ of castor oil (filled symbols), a decrease of the aroma is noted until complete depletion, but this was not observed when a concentration of 60 $g{\cdot}L^{-1}$ of castor oil was used, in which a higher concentration of aroma still remained in the medium. Braga *et al.*[33] investigated the influence of different castor oil concentrations (10 $g{\cdot}L^{-1}$, 30 $g{\cdot}L^{-1}$ and 50 g L^{-1}) on γ-decalactone production in flask experiments, and concluded that the best substrate concentration was 30 $g{\cdot}L^{-1}$ of castor oil, obtaining an aroma production of 1.8 $g{\cdot}L^{-1}$. An *et al.*[18] also studied the influence of different substrate concentrations on the production of γ-dodecalactone by permeabilized *Waltomyces lipofer* cells. They observed that increasing the substrate concentration resulted in proportional increases in the production of γ-dodecalactone. However, the production reached

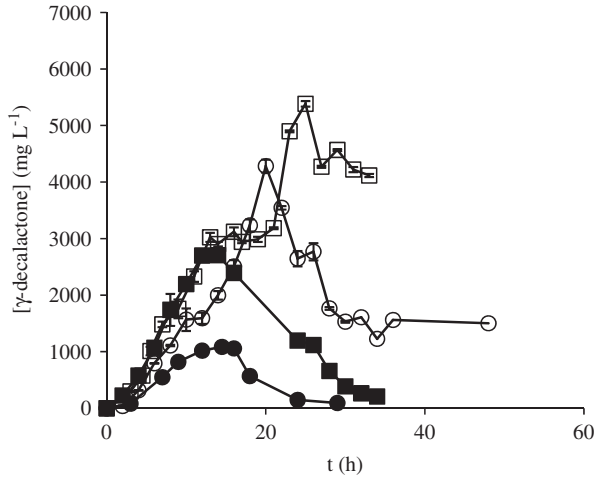

Figure 4. Effect of cell concentration and substrate concentration in γ-decalactone production: (●) 30 g·L^{-1} cell and 30 g·L^{-1} of CO; (■) 60 g·L^{-1} cell and 30 g·L^{-1} of CO; (□) 60 g·L^{-1} cell and 60 g·L^{-1} of CO and (○) 30 g·L^{-1} cell and 60 g·L^{-1} of CO. Data are presented as the mean and standard deviation of two independent experiments.

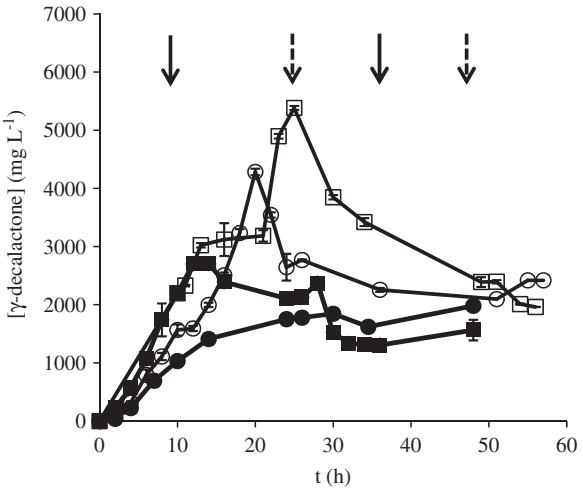

Figure 5. γ-Decalactone production in a step-wise fed-batch for (●) 30 g·L^{-1} cell and 30 g·L^{-1} of CO; (■) 60 g·L^{-1} cell and 30 g·L^{-1} of CO; (□) 60 g L^{-1} cell and 60 g L^{-1} of CO and (○) 30 g L^{-1} cell and 60 g L^{-1} of CO. The solid arrows indicate 30 g L^{-1} CO addition to the medium (experiments with filled symbols) and dashed arrows indicated 60 g L^{-1} CO addition to the medium (experiments with opened symbols). Data are presented as the mean and standard deviation of two independent experiments.

a plateau for substrate concentrations higher than 60 g·L^{-1}. Similar results were also reported by Dufossé *et al.*[17] who showed that γ-decalactone concentration obtained in the culture broth of *Sporidiobolus ruinenii* increased almost proportionally with ricinoleic acid methyl ester concentration between 0 and 160 g·L^{-1}. Our results contrasted with those reported by Alchihab *et al.*[16] who observed that γ-decalactone concentration obtained with the strain *Rhodotorula aurantiaca* was not proportional to the concentration of castor oil added. After 10 days in flasks, the highest γ-decalactone production, 5.5 g·L^{-1}, was recorded with 20 g·L^{-1} of castor oil. The production was reduced for higher castor oil contents (30, 40, 50 and 60 g·L^{-1}). Similar results have also been reported by Endrizzi *et al.*[34] who observed that a high concentration of ricinoleic acid methyl ester lowers the yield of γ-decalactone produced by *Pichia guilliermondii*.

In experiments with 30 g·L^{-1} of castor oil, a 2-fold increase in cell concentration leads to a 2.2-fold enhancement in lactone production (1.2 ± 0.1 g·L^{-1} and 2.7 ± 0.4 g·L^{-1}, for 30 g·L^{-1} and 60 g·L^{-1} cell concentration, respectively). An *et al.*[18] conducted experiments varying cellular concentrations (0–50 g·L^{-1}) and analyzed its impact on γ-decalactone production by permeabilized *Waltomyces lipofer* cells. They observed that at concentrations less than 30 g·L^{-1} of permeabilized cells, γ-dodecalactone production increased as the concentration of permeabilized cells increased; however, at concentrations higher than 30 g·L^{-1} γ-dodecalactone production reached a plateau, determining the optimal cell concentration at 30 g·L^{-1}.

Step-wise fed-batch culture

Yarrowia lipolytica can use γ-decalactone as a carbon source[35] resulting in its complete disappearance after some hours of batch culture, as previously observed in the experiments with lower oil concentration (Fig. 4). However, although this is avoided with an increase in the substrate concentration, in the expectation to achieve higher γ-decalactone productivities, a step-wise fed-batch strategy was attempted in which castor oil was added in pulses to the bioreactor (Fig. 5).

Figure 5 shows that in a step-wise fed-batch culture the concentration of γ-decalactone did not disappear from the medium as occurred in batch cultures for the lower oil concentrations. For the experiments performed with 30 g·L^{-1} of castor oil a maximum concentration of 2.7 ± 0.4 g·L^{-1} of γ-decalactone was obtained after 14 h (60 g·L^{-1} cell and 30 g·L^{-1} of castor oil). Moreover, after feeding fresh medium, the aroma slightly decreased but maintained a constant value in the biotransformation medium during the whole experiment. However, the substrate additions in experiments performed with 60 g·L^{-1} of substrate did not enhance aroma production, achieving similar production with those obtained in batch experiments (Fig. 4) and contrasting with the observations in experiments with 30 g·L^{-1} of castor oil. This fact reinforces the hypothesis that the γ-decalactone measured resulted from an equilibrium between production and consumption of the aroma. Another possible explanation is that β-oxidation is not completely stopped at the C_{10} level (Fig. 1) because, when higher substrate concentrations were presented the C_{10} product may be used rather as precursor for other lactones,[35] such as 3-hydroxy-γ – decalactone,[5] and γ-decalactone accumulation was reduced. Nevertheless, γ-decalactone production reached its maximum for the higher castor oil concentration (60 g·L^{-1}) present at the beginning of the fermentation, rather than when added separately at a later stage, showing that for the substrate concentration analyzed no substrate inhibition took place.

Table 2. Maximum productivity of γ-decalactone, obtained in bioreactor, under different operating conditions. Data are presented as the mean and standard deviation of two independent experiments

Strategy	Cell concentration (g·L^{-1})	Productivity (mg·L^{-1}·h^{-1}) Castor oil concentration (g·L^{-1}) 30	60
Batch	30	75 ± 13	214 ± 18
	60	193 ± 21	215 ± 19
Step-wise fed-batch	30	41 ± 10	214 ± 15
	60	193 ± 21	215 ± 13

In general, the productivity values obtained with step-wise fed-batch and batch approach where quite similar.

No greater improvement in γ-decalactone production was achieved using step-wise fed-batch and the batch strategy is preferable. Although the γ-decalactone concentration achieved with the step-wise fed-batch strategy was lower than the value obtained by Gomes *et al.*[21] (6.7 g·L^{-1}), we achieved a much higher productivity (215 ± 13 mg L^{-1}·h^{-1} vs 42.5 mg·L^{-1}·h^{-1}).

Nevertheless, most of the papers reported an increase in γ-decalactone production in fed-batch when compared with batch.[20] Lee *et al.*[20] attempted a fed-batch strategy to produce γ-decalactone by *Sporobolomyces odorus* from castor oil hydrolysate as source of ricinoleic acid. High aroma production was also described for three-step feeding of castor oil, in which a maximum concentration of γ-decalactone, 208 mg·L^{-1}, was obtained over 168 h. Ambid *et al.*[36] studied the γ-decalactone production with the same yeast, with the goal of increasing the process productivity, methyl ricinoleate additions with 24 h gaps were tested, and a considerable improvement in aroma production was found (142.7 mg·L^{-1} instead of 47.7 mg·L^{-1}) in a fed-batch operation.

The results presented are very promising for γ-decalactone production, showing that higher initial castor oil concentrations (60 g·L^{-1}) can be used in batch cultures instead of lower substrate step-wise feeds.

CONCLUSIONS

γ-Decalactone production from castor oil by *Y. lipolytica* W29 has been studied taking into account the effects of oxygen transfer rate, substrate and cellular concentration and mode of operation – batch and step-wise fed-batch.

The direct influence of oxygen transfer rate on the γ-decalactone production was demonstrated. Also, castor oil and cellular concentration of 60 g·L^{-1} were shown to be the most adequate conditions for lactone production in batch cultures. In addition, with higher substrate concentration (60 g·L^{-1}) it was possible to prevent aroma depletion. The productivity values obtained with batch and step-wise fed-batch approach where quite similar, but no greater improvement in γ-decalactone concentration was achieved using step-wise fed-batch than batch strategy. Thus, no castor oil toxicity to cells was found for 60 g·L^{-1} in batch culture, showing that this is the most adequate strategy for γ-decalactone production by *Y. lipolytica* W29.

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