

Production of γ -Decalactone through Fermentation: A Review

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Abstract

γ -Decalactone (Chemical formula: $C_{10}H_{18}O_2$) is an important aroma flavor compound and has peachy fruit odor. It is approved as GRAS food additive by FDA and generally present in the cultures of *Sporobolomyces odoratus*. This review focus on the pathways, metabolic engineering strategies and bioengineering problems to enhance γ -decalactone production by taking the consideration of toxicity, degradation of produced lactone and oxygen transfer problems.

Keywords: γ -Decalactone, Production strategies, Yeast strains and Fermentation

I. Introduction

Flavors and fragrances have extensive applications in feed, food, chemical, cosmetic and pharmaceutical industries [1]. Many flavor compounds in the current market are producing through chemical synthesis or extraction from plants. However, due to high cost, lack of natural availability of extracts and unfriendly environmental production processes, increase in health-conscious life styles and chemophobia-attitude towards synthetic compounds, the demand for biotechnological production of flavors and fragrances has been growing nowadays [2-4].

There are certain disadvantages for natural production of flavor compounds by extraction methods such as low product concentration, dependency on climatic and seasonal changes and ecological problems connected with extraction. An alternative process to overcome these problems is biotechnological production process and these occurs at atmospheric conditions, environmentally friendly and products may be labelled as natural. The biotechnological route for synthesis of flavor compounds is based on *de novo* process or bioconversion of precursors with microbial cells or enzymes. However, product concentrations are very low and thus constitute a major limit for industrial exploitation. For this reason, biotechnologists have focused on bioconversion process that offer more yield and economic advantages [5]. Recent studies revealed that the use of genetic engineering approaches to develop high novel yielding strains, adoption of bioengineering techniques and use of agro-industrial residues can enhance the yield of flavor compounds.

II. LACTONES

Lactones are industrially important volatile compounds and some of them include γ -decalactone, δ -decalactone, γ -undecalactone and β -methyl- γ -octalactone and others. Among lactones, γ -Decalactone has a pleasant fruity peach odor with an aroma threshold between 1 to 11 ppb. It can be detected in water

concentrations as low as 0.08 ppm. Currently, it is producing in highest volume among all lactones through biotechnological process. The price of γ -decalactone reduced to 300 US dollar per Kg from 1.2 thousand US dollars per Kg in 1986 with the optimization of biotechnological processes.

III. MICROORGANISMS

There are various microorganisms chosen for their potentialities to produce aroma, in which the most important are *Pseudomonas*, *Sporobolomyces*, *Pichia*, *Candida* and *Rhodotorula*,

being *Yarrowia lipolytica* species the one with a higher productivity.

Sporidiobolus salmonicolor is a yeast like fungus commonly used to produce γ -decalactone. It is considered as Biosafety Risk Group 1 fungus [6]. Three kinds of *Sporidiobolus salmonicolor* have been described and these are *Sporidiobolus salmonicolor* var. *albus*, *Sporidiobolus salmonicolor* var. *fischerii*, and *Sporidiobolus salmonicolor* var. *salmonius* [7]. The isolates of *Sporidiobolus salmonicolor* are obtained from cerebrospinal fluid, infected skin, a nasal polyp and lymphadenitis.

Yarrowia lipolytica is another yeast strain used for conversion of ricinoleic acid to γ -decalactone and which is used to obtain higher yields due to its ability to grow on hydrophobic substrates and due to their efficient lipases, several cytochrome P450s, and acyl-CoA oxidase [8].

IV. SUBSTRATES

Castor oil is used as main substrate in most production processes of γ -decalactone. It contains 86% of ricinoleic acid (12-hydroxy-octadec-9-enoic acid). Ricinoleic acid transforms into γ -decalactone by yeast strains through peroxisomal β -oxidation pathway [9]. Another substrate used in the production processes of γ -decalactone is crude glycerol due to its readily availability and cost effectiveness [10,11].

V. CULTURE CONDITIONS

In addition to carbon sources, pH, DO and agitation speed of medium also significantly affects γ -decalactone production. The values of oxygen mass transfer rates can be improved by increase in aeration and agitation rates since the oxygen influence the activity of enzymes in peroxisomal β -oxidation pathway [12].

VI. γ -DECALACTONE ANALYSIS

A two mL of γ -decalactone was extracted from conversion medium with 2 mL of diethyl ether by ten gentle shakings. After 5 min, ether phase was separated and analyzed by gas chromatography as described by [12].

VII. γ -DECALACTONE PATHWAYS

The γ -decalactone pathway was first discovered by Okui et. al. 1963 [13] during the studies of hydroxyacids metabolism from several organisms. After Okui's investigation many other research groups have been exploring this topic. Among them, Endrizzi-Joran et al. 1993 discovered the β -oxidation pathway & it is classical biochemical route involved in fatty acid degradation [14]. Feron et al. 1996b studied toxicity levels of this lactone [15], while Endrizzi-Joran, 1993 reported the degradation of γ -decalactone [14]. Waché and co-works explored the influence of acyl-CoA enzymes in the conversion of methyl ricinoleate into γ -decalactone by *Yarrowia lipolytica* with the aim of enhancing its production [16]. Recent studies have focusing on gene function that encodes acyl-CoA oxidase isozymes, lipid metabolism, oxygen mass transfer rates, selection of over producing mutants, interactions of cell- substrate to increase production of γ -decalactone.

VIII. PRODUCTION PROCESSES

After some hours during batch fermentation, yeast cells consume γ -decalactone as carbon source when substrate is completely exhausted. Thus, results in the complete disappearance of decalactone from the medium [17,18]. Production rates depends on the difference between the growth and consumption rates. An alternative method to

overcome this problem is fed-batch operation in the which substrate is supplied intermittently or continuously when substrate falls below the desired level. This operation allows us to obtain higher yields and productivities of desired product by controlling the nutrient media [19].

The γ -decalactone production of 512.5 mg/L by *L.saturnus* CCMA 0243 (pH-5.0) and 214.8 mg/L by *Y. lipolytica* CCMA 0242 (pH-6.0) at 96 h of fermentation in fed-batch fermentation at 30% castor oil reported by [9]. After 96 h production of γ -decalactone by yeast strains decreased due to variation in oxygen concentration which is directly linked to activity of various enzymes involved in the metabolic pathway results in the production of other lactones.

A lactone of coconut flavor, 6-pentyl- α -pyrone, was produced by a soil fungus, *Trichoderma viride*, reaching 170 mg/L [20]. The highest γ -decalactone production of 220 mg/L was achieved in fed-batch fermentation using pure oxygen by *Yarrowia lipolytica* which is threefold more compared with batch cultivation [21].

As per the study of γ -decalactone productions reported by various authors, conversion levels of ricinoleate to γ -decalactone are very low and mainly due to degradation of lactone and formation of other lactones during the β -oxidation pathway.

CONCLUSIONS

In the last four decades, even after thorough analysis of metabolic pathways of γ -decalactone and couldn't able to obtain high conversion levels. This is mainly due to incomplete conversion of ricinoleate to γ -decalactone, degradation of γ -decalactone and formation of other lactones such as 3-hydroxy- γ -decalactone and 2- and 3-decen-4-olide. Hence, there is need to conduct more studies on decalactone pathways and generation of mutant strains with no activity of acyl-CoA enzyme to reduce reconsumption of γ -decalactone.

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