Process Biochemistry 51 (2016) 1479–1485

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Optimization of the lipase mediated epoxidation of monoterpenes using the design of experiments—Taguchi method

Sumanth Ranganathan [a], Johannes Tebbe [b], Lars O. Wiemann [b,1], Volker Sieber [a,b,*]

[a] Chair of Chemistry of Biogenic Resources, Technical University of Munich, Schulgasse 16, Straubing 94315, Germany
[b] Fraunhofer Institute of Interfacial Engineering and Biotechnology (IGB) Bio-, Electro and Chemo Catalysis BioCat Branch Straubing, Schulgasse 11a, Straubing 94315, Germany

ARTICLE INFO

Article history:
Received 12 May 2016
Received in revised form 24 June 2016
Accepted 5 July 2016
Available online 6 July 2016

Keywords:
Epoxidation
Lipase
Design of experiments
Taguchi method
Monoterpenes
Process optimization

ABSTRACT

This work deals with the optimization of the *Candida antartica* lipase B (CALB) mediated epoxidation of monoterpenes by using the design of experiments (DoE) working with the Taguchi Method. Epoxides are essential organic intermediates that find various industrial applications making the epoxidation one of the most investigated processes in chemical industry. As many as 8 parameters such as the reaction medium, carboxylic acid type, carboxylic acid concentration, temperature, monoterpene type, monoterpene concentration, hydrogen peroxide concentration and amount of lipase were optimized using as less as 18 runs in triplicates (54 runs). As a result, the hydrogen peroxide concentration used was found to be the most influential parameter of this process while the type of monoterpene was least influential. Scaling up of the reaction conditions according to the findings of the optimization achieved full conversion in less than 6 h. In addition, a purification process for the epoxides was developed leading to an isolated yield of ca. 72.3%, 88.8% and 62.5% for α-pinene, 3-carene and limonene, respectively.

1. Introduction

Epoxides possess high polarities and ring strains making them a highly reactive species and very useful building blocks in organic synthesis. They are predominantly synthesized with the Prileschajew epoxidation method using peroxycarboxylic acids, that in turn attack the double bonds of alkenes [1,2]. Peroxycarboxylic acids are extremely reactive, possess high oxidation potentials and are therefore recommended to be produced *in-situ* for safe operation of the epoxidation process [3]. The most commonly used substance for Prileschajew epoxidation is *meta*-chloroperbenzoic acid – a strong electrophile prone to detonation when exposed to shocks in the environment. In addition to the explosive nature, these reactions should be performed at a temperature range of 0–25 °C [4].

Owing to the aforementioned operational hazards of using high amounts of this substance and the subsequent cleaning steps involved thereafter, chemo-enzymatic *in-situ* generation of peroxycarboxylic acids was developed by Fredrik Björkling and his co-workers in the early 1990s using lipases (glycerol ester hydrolases, E.C. 3.1.1.3). The process (Scheme 1 [5]) was the first of its kind and subsequent works have been carried out using this protocol [6–10]; to name a few. Variations of this process have been reported by the works of Ankudey et al. [11], when they used ethyl acetate as the solvent and acid donor for the epoxidation process. Another modification of the Bjökling process was carried out by Klass & Warwel [12], where the researchers used dimethyl carbonate to epoxidize alkenes and carbon dioxide was obtained as the by-product. In addition to this, Baeyer Villiger Oxidation has also been done using the mechanism explained by Björkling and his co-workers [13–15].

Every process needs to be optimized for good yields and the process shown in Scheme 1 is no exception. On optimizing this process at a small scale (laboratory and pilot), the industrial production could be achieved with pure products being formed and less waste being generated. The outcome of an experiment highly depends on the careful design of the experimental process [16]. Generally, in the design of a statistically based experiment the first step is the choice of the performance characteristic or the response variable, which will be closely monitored. The second step is the identification of variables or factors that contribute to this response variable, which will be studied. The next step is the choice of different treatment stages or levels, at which these factors will be tested for individual experiments. The final step is the identification of uncontrollable factors or noise factors that may influence the process in any way [17]. The usage of statistical procedures

* Corresponding author at: Chair of Chemistry of Biogenic Resources, Technical University of Munich, Schulgasse 16, Straubing 94315, Germany.
E-mail address: sieber@tum.de (V. Sieber).
[1] Current address: evocatal GmbH, Alfred-Nobel-Str. 10, Monheim am Rhein 40789, Germany.

http://dx.doi.org/10.1016/j.procbio.2016.07.005
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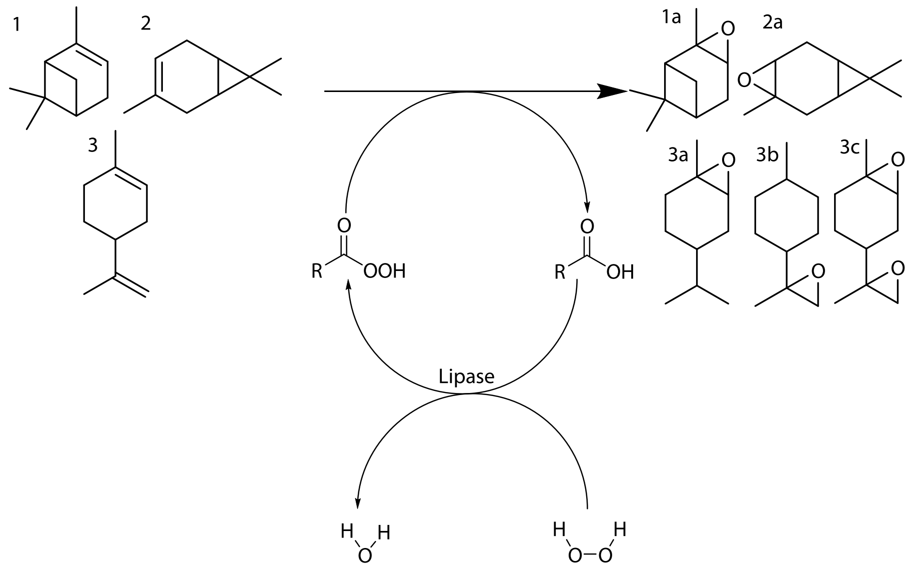

Scheme 1. Lipase mediated epoxidation of monoterpenes using peroxycarboxylic acids according to the method of Björkling et al. [5]. (1-α-pinene, 2–3-carene and 3-limonene; 1a-α-pinene epoxide, 2a-3-carene epoxide, 3a and b-limonene monoepxoide and 3c-limonene diepoxide).

follows the general principles of randomization, replication and duplication to predict the actual behavior of a process. Generally, Plackett-Burmann Design (PBD), Central Composite Design (CCD) and Box-Benkhen Design (BBD) have already been used to optimize several processes.

The optimization of the above mentioned lipase mediated epoxidation of alkenes has already been carried out with the traditional 'alteration of one variable at a time' [18,19] and also using the response surface methodology approach [20,21]. The disadvantage of the one variable at a time approach is that it generates large amounts of samples and waste, is extremely time consuming and also expensive. Although, the response surface methodology system is advantageous in minimizing the number of trials and predicting interactions of the variables used, the Taguchi method with orthogonal array design predicts a mean performance characteristic value close to the target value, instead of just adhering to traditional limits, which in turn improves the quality of the process/product [22]. The present work deals exclusively with the optimization of this lipase mediated epoxidation process for such monoterpene substrates, esp. α-pinene, 3-carene and limonene using the Taguchi approach. Once the process has been tested for these three substrates, the procedure will be expanded to other terpenes and alkenes as well. Monoterpenes are simple plant products that are found predominantly in essential oils, but also in waste streams of pulp and paper industries and are widely used in the food, paint and pharmaceutical industries. Their oxygenated versions, viz. monoterpene epoxides and the corresponding diols are building blocks and synthetic intermediates [10]. Another important aspect to consider is that in classical chemical epoxidation approach, various unwanted side products are generated [23]. Seven parameters at three different settings and one parameter at two different settings were tested for obtaining the maximum conversion. Scale-up of the optimized runs obtained from Taguchi method was investigated and found out to comply with the results.

2. Materials and methods

2.1. Introduction—Taguchi method of experimental design

Many of the industrial processes of today use the technique that was developed by Dr. Genichi Taguchi [24]. The Taguchi method was developed on the foundations of robust design introduced in the 1950s and 1960s. Robust design can be defined as *"an engineering methodology for improving productivity during research and development so that high-quality products can be produced quickly and at low cost"*. This method can be applied to a range of problems and has already been used in the field of electronics, automotives, photography and many others [25].

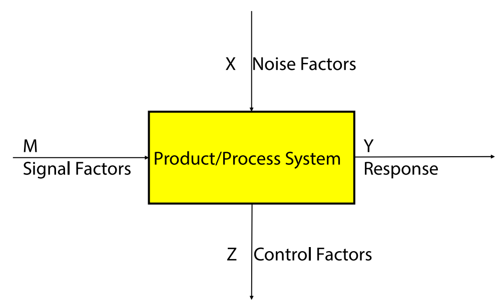

Fig. 1. Parameter diagram for product/process system.

On designing a process based on robustness strategy, the following approach is to be followed:

- Drafting of the P-diagram and classification of variables into noise (uncontrollable), signal (input) and response (output) factors (Fig. 1).
- Use of orthogonal arrays for gathering usable information about the control factors by carrying out a minimal amount of experiments.
- Determination of signal to noise ratio for determining the field quality through laboratory experiments. Because, with a decreasing mean, the standard deviation also decreases. The standard deviation cannot be reduced first and mean brought to the target value [26]. Hence, the signal to noise ratio is used.
- Use of this ratio in the specified way (larger the better, minimal the better and nominal the best) to determine the outcome of the process.