HIGHLIGHTS

- The environmentally-friendly biosynthesis of adipic and sebacic diesters is studied
- The evaporation of 2-ethyl-1-butanol is addressed by different reaction strategies
- The use of a fixed-bed reactor led to concentrations of 20 – 30% (w/w) on diester
- Product concentrations of 75% (w/w) were obtained by using a fed batch reactor
- High purity products were achieved with 2-ethyl-1-butanol excess in a batch reactor

Sustainable synthesis of branched-chain diesters

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ABSTRACT

Esters from branched alcohols and dicarboxylic linear acids are widely used as lube bases due to their good performance at low temperatures. This work proposes a new process to synthesize bis(2-ethylbutyl) adipate and bis(2-ethylbutyl) sebacate by using the lipase-based catalyst Novozym® 435 in a solvent-free system. Different reaction strategies have been tested in order to minimize 2-ethyl-1-butanol losses due to its evaporation and optimum operation conditions have been determined: 2.5% of biocatalyst, 50 ºC and a molar excess of alcohol of 15% for the adipic diester and of 25% for the sebacic one. It has also been proven that the immobilized enzyme can be reused in seven successive reaction cycles, achieving high yields without an appreciable reduction of activity. This biocatalytic pathway is a promising basis for the development of a more sustainable large scale process for obtaining biodegradable lubricants, as it is pointed out by productivity, economic and green metrics calculations.

KEYWORDS: lipase, solvent-free, biolubricants, bis(2-ethylbutyl) adipate, bis(2-ethylbutyl) sebacate.

ABBREVIATIONS

BEBA: Bis(2-ethylbutyl) adipate

BEBS: Bis(2-ethylbutyl) sebacate

DMA: Dimethyl adipate

DES: Diethyl sebacate

EB: 2-Ethyl-1-butanol

MEBA: Methyl 2-ethylbutyl adipate

EEBS: Ethyl 2-ethylbutyl sebacate

1. INTRODUCTION

The lubricant industry is in clear evolution. It has gone from being a traditional sector to an industry that pursues to obtain products increasingly adapted to particular applications. Traditionally, lubricants were based on mineral or vegetable oils, but the severe requirements of new machinery can cause the decomposition and release to the environment of the former and problems with the oxidative and hydrolytic stability of the latter (Bried et al., 1947; Narayan and Madras, 2017). This need of chemically stable lubricants with a good performance under liquid form in a wider range of temperatures has derived in the development of synthetic lubricants with custom properties (Akerman et al., 2011). Therefore, most of the producers have introduced a synthetic or semi-synthetic variant in their catalogue.

Besides, the general concern for the environment that has arisen during the last years has also pushed lubricant manufacturers to design biodegradable formulations. So that, nowadays five groups of biodegradable compounds with interesting prospects as lube bases can be found: high oleic vegetable oils, polyalphaolephins, polyalkylene glycols, polyol esters and dicarboxylic acid esters (Nagendramma and Kaul, 2012). Due to their shear, thermal and oxidation stability, diesters stands out for providing better technical features than common mineral or vegetable oils, but they are more expensive. Among them, adipic and sebacic diesters are the most used (Gryglewicz and Oko, 2005; Nagendramma and Kaul, 2012).

As previously stated, the synthetic origin of dibasic esters allows the selection of the most suitable properties for lubricant application: low pour point, high viscosity index and flash point, resistance to corrosion and high oxidation stability (Zainal et al., 2018). Particularly, esters from branched alcohols and straight dicarboxylic acids have been subject of several studies on their physicochemical properties in order to be used as lube base stocks. In this sense, it has been observed that the linear diacid part provides excellent viscosity indexes but high pour points, while the branched alcoholic part contributes to lower this last property (Comuñas et al., 2008). In general, the branching of the alcohol provides a better performance of the ester at low temperatures because it contributes to reduce its freezing point, but it may diminish their resistance to oxidation and biodegradability. In addition, the presence of the ester bounds confers to branched diesters low volatility, a better solubility and high flash point (Bried et al., 1947; Gryglewicz and Oko, 2005; Nagendramma and Kaul, 2012), but it makes them susceptible of hydrolytic decomposition. Nevertheless, a higher stability can be reached by placing one or more short chained branches close to the ester group in order to prevent the action of nucleophiles by steric hindrance (Bried et al., 1947). Because of

the good extreme temperature performance of these type of esters, bis(2-ethylbutyl) adipate (BEBA) and bis(2-ethylbutyl) sebacate (BEBS) can be potentially used as plasticisers, hydraulic fluids, engine and compressor oils or white oils in food and textile industries (Gryglewicz and Kolwzan, 2004).

So far, the large-scale production of esters (not only for lubricant use, but also for cosmetics, plastizers, etc.) is mainly based on the use of acid catalysts, usually sulphuric acid. This synthetic pathway gives rise to high energetic requirements and to the need for a host of auxiliary procedures due to the undesired side reactions and the acidic wastewater generation. It also entails technical and economic efforts in order to avoid the equipment's corrosion, the workers exposure to dangerous chemicals or the difficulties for recovering the catalyst from reaction mixture (Gryglewicz, 2000; Narayan and Madras, 2017; Serrano-Arnaldos et al., 2018). As a consequence, new investments for more efficient and environmentally friendly processes are being made, like enzymatic catalysis (Madarasz et al., 2015). In this regard, lipases are a group of enzymes that can catalyse esterification (Bansode et al., 2017; El-Boulifi et al., 2014), interesterification (Lei et al., 2016; Otero et al., 2012) or alcoholysis (Babaki et al., 2016; Mangas-Sánchez et al., 2015) reactions, providing medium and long-chain esters of high purity under mild operating conditions. While esterases require higher water activities (a_w) and short-chain esters to develop their catalytic activity, lipases are active in practically anhydrous media and without using a solvent, i.e., under solvent-free conditions. Concretely, *Candida antarctica* lipase B (CalB) can display great activity at low a_w and does not show interfacial activation (Adlercreutz, 2013; Kapoor and Gupta, 2012; Bracco et al., 2020; Norblad and Adlercreutz, 2013). Thus, the main advantage of using solvent-free systems with immobilised lipases is that, when high degrees of conversion are achieved, products require minimum downstream processing (Thum and Oxenbøll, 2008).

Even if their great utility is known since 1947 (Bried et al., 1947), to the best of our knowledge, a sustainable enzymatic procedure to obtain BEBA and BEBS has not been previously described. So, in view of the foregoing, the aim of this work is the optimization of synthesis the adipic and sebacic esters of 2-ethyl-1-butanol, catalysed by a commercial immobilized lipase in a solvent-free medium (Figure 1) through different reaction strategies.

(FIGURE 1)

2. MATERIALS AND METHODS

2.1. Materials

The biocatalyst used was Novozym® 435, a commercial *Candida antarctica* lipase B immobilised on a macroporous acrylic resin, which was kindly donated by Novozymes Spain S.A.

Substrates dimethyl adipate (≥ 99 %), diethyl sebacate (98 %) and 2-ethyl-1-butanol (98 %) were purchased from Sigma-Aldrich® (the selection of diethyl sebacate instead of dimethyl sebacate as substrate was based only on economic criteria). As final products cannot be purchased, bis(2-ethylbutyl) adipate (> 99%) and bis(2-ethylbutyl) sebacate (> 96%) were obtained in-lab by following the experimental procedure described in the subsequent section. The syntheses of BEBA and BEBS were allowed to evolve for 24 – 48 hours and product was characterized by nuclear magnetic resonance (the corresponding spectra can be consulted in the supplementary material).

Methyl myristate (≥ 99%) from Sigma-Aldrich was used as internal standard for the gas chromatography analysis of the samples. Other chemicals were analytical reagent grade.

2.2. Diester synthesis reaction in tank reactors

The alcoholysis reactions were carried out in a 250 mL glass-jacketed open-air tank reactor provided with vertical stirring (two bladed propeller set at 350 rpm).

Several standard experiments were performed in a solvent-free reaction system composed by 20 g of reagents in a molar ratio of 1:2 (DMA:EB or DES:EB), where reaction temperature was set at 50 or 60 ºC. Once the reaction mixture was homogeneous, the biocatalyst was added and concentrations of 1.25%, 2.5% y 5% (w/w referred to substrates) were assayed. Two additional experiments were performed in order to study of the stepwise addition of EB, where five equal amounts of this substrate were added to reach a final molar ratio of 1:2. Also, the one-step process using reagent molar ratios of 1:2.3 y 1:2.5 (DMA:EB or DES:EB), which corresponds to a EB molar excesses of 15 and 25% respectively, was tested. The substrates quantities used in these different sets of experiments are specified in Table 1.

Reaction course was followed by withdrawing samples along the experiments (stirring was stopped a few seconds before to let the biocatalyst sediment), which were dissolved in absolute ethanol in order to allow their GC analysis by an internal standard method. Several reactions were performed in duplicate, observing standard deviation ≤10%.

Table 1. Substrates quantities and EB addition times for the experiments performed in the batch reactor.

	Standard experiments 1:2 molar ratio	1º stepwise experiment with 5 additions	2º stepwise experiment with 5 additions	Experiment with an excess of 15% of EB	Experiment with an excess of 25% of EB
DMA (g)	9.20	9.20	9.20	8.51	8.11
EB (g)	10.80	5 × 2.16 *(0, 60, 120, 180 and 240 min)*	5 × 2.16 *(0, 30, 90, 180 and 300 min)*	11.49	11.89
DES (g)	11.17	8.83	8.83	10.47	10.06
EB (g)	8.83	5 × 1.77 *(0, 60, 120, 180 and 240 min)*	5 × 1.77 *(0, 30, 90, 150 and 240 min)*	9.52	9.95

2.3. Diester synthesis in packed bed reactors

The diesters under study were also synthesised in a glass-jacketed tubular reactor (21 cm high, 1.1 cm internal diameter) provided with a sintered glass plate located at 3.5 cm from the base. An amount of biocatalyst of 0.5 g was introduced in the reactor and 20 g of reagents with molar ratio 1:2 (DMA:EB or DES:EB) were recirculated (downflow) by means of inert silicone tubes and a peristaltic bomb Watson Marlow 505 DU, at 0.09 mL s^{-1} during the first two hours and at 0.04 mL s^{-1} afterwards. Reaction temperature was of 50 ºC and sampling was performed as described in previous section.

2.4. GC analysis

The content on reagents and products was determined by injecting 1 µL of diluted sample in a 7820A gas chromatographer from Agilent, equipped with a flame ionization detector and a capillary column HP-5 Agilent Technologies (30 m × 0.32 mm × 0.25 µm). Injector's temperature was set at 250 ºC, with a split ratio 2:1 and 1 mL min^{-1} of nitrogen was used as carrier gas. Oven start temperature was 80 ºC, which was held for a minute and increased to 120 ºC with a rate of 75 ºC min^{-1}, which was also maintained for a minute. Then, oven's temperature was increased to 290 ºC at a ramping rate of 20 ºC min^{-1}, temperature which was held for 3.5 min. Detector's temperature

was 300 ºC. The samples composition was determined by using of methyl myristate as the internal standard (IS).

3. RESULTS AND DISCUSSION

3.1. Preliminary studies

As far as authors know, the enzymatic synthesis of the two diesters under study (BEBA and BEBS) has not been described in the bibliography yet. For our purpose, CalB biocatalyst Novozym® 435 was selected, as many studies have proven its robustness and unparalleled catalytic activity (Ortiz et al., 2019). Thus, a proof of concept test was performed with the aim of determining if this commercial immobilized derivative was able to catalyze the reaction between adipic and sebacic acids and EB.

Unlike other research work dealing with the direct esterification of adipic acid with longer alcohols (Kim et al., 2019; Lee et al., 2019), during this preliminary assay, a lack of solubility of the two solid dicarboxylic acids in EB has been observed (results not shown). This fact, added to the acids' high fusion point, made evident that the solvent-free biocatalytic synthesis of BEBA and BEBS should be carried out by means of an alcoholysis reaction instead the methodology previously described in the bibliography. As a consequence, the authors opted to use the methyl and ethyl esters of adipic and sebacic acid as substrates, even if the release of carbonated alcohols (methanol and ethanol) to the atmosphere could be a clear drawback of this enzymatic pathway, as it has a negative impact on one of the most important metrics used, among others, to measure the sustainability of a process: the carbon mass efficiency (CME) (Lima-Ramos et al., 2014). In this sense, according to the stoichiometry of the process, if a complete transformation of substrates into products would be achieved, ~17% of methanol and ~20% of ethanol (w/w referred to the total amount of substrates) will be liberated to the atmosphere, instead of the ~10% of water that would have been released if solvent-free esterification would have been feasible. On the other hand, removing methanol or ethanol as the by-product is easier than removing water due to their lower boiling point, which clearly helps pushing the reaction equilibrium towards product formation.

Considering this, both preliminary alcoholysis experiments were carried out by following the procedure explained in *Diester synthesis reaction in tank reactors* section, with a substrates molar ratio of 1:2 (DMA: EB or DES:EB), 2.5% (w/w) of biocatalyst and 60 ºC. Only three samples were withdrawn, as they were considered enough to prove the hypothesis that CalB can catalyze the process. Substrates and final products BEBA and BEBS were quantified by their respective calibration lines, obtained as

described in *Materials* section. Regarding the intermediate products, MEBA and EEBS were estimated by difference from the total mass injected.

Results for these initial assays are depicted in Figure 2, where it has been represented how the concentration of the four substances present in reaction media changes with time. As it can be observed, both intermediate and final products are obtained since the beginning of the assay and the evolution of all the chemical species accurately follows what is expected for reactions whose kinetics are dependent of an intermediate product, as it is the case with the reactions studied. Comparing Figure 2 A with B, it becomes apparent that the formation rate of BEBA is faster than BEBS' and after 6 hours, higher concentrations of the adipic diester are found in reaction medium, pointing out the importance of the size of the dicarboxylic acid used (C6 for adipic acid, C10 for sebacic). These observations are in line with the bibliography dealing with the structure of CalB, which have been described as a lipase with a relatively narrow acyl binding cleft (Pleiss et al., 1998; Uppenberg et al., 1994).

Once the technical viability of the enzymatic processes was confirmed, reaction conditions optimization was initiated.

(FIGURE 2)

3.2. Influence of biocatalyst concentration and temperature.

The quantity of immobilized enzyme used in a biocatalytic process is of paramount importance when optimizing it. As a general trend, when the available enzyme in the reaction medium is increased, the reaction rate is faster and less time is needed to attain the desired conversion; hence, cost operation can be reduced (Serrano-Arnaldos et al., 2019). However, using an excessive amount of biocatalyst has a negative impact not only on the economy of the biosynthesis, but also on the reaction process in itself due to the diffusional mass transfer limitations. Accordingly, the influence of biocatalyst concentrations of 1.25%, 2.5% and 5% (w/w, referred to substrates) has been tested in the production of BEBA and BEBS. Results are shown in Figure 3, from which it can be verified that final product concentration in BEBA is higher than 80%, while it is lower than 75% for BEBS for the same reaction time. Such observations confirms that obtaining the diester from adipic acid with immobilized CalB is more favorable than the diester from sebacic acid, as it has already been discussed in the previous section.

As it can also be noticed in Figure 3, the alcoholysis process is clearly enhanced by increasing biocatalyst concentration from 0.75% to 2.5%, but no great improvement is achieved either on final concentration or on total reaction time when biocatalyst amount is doubled again. Concentrations lower than 0.75% were not tested in order not to

unduly lengthen the reaction time. In any case, those values are lower than the ones described by other authors, who carried out the synthesis of adipic and sebacic esters by using concentrations between 5 and 10% (w/w) (Chaibakhsh et al., 2009; Gryglewicz, 2003; Kim et al., 2019; Lee et al., 2019). Thus, a concentration of immobilized derivative of 2.5% was selected to perform the remaining assays.

(FIGURE 3)

Another factor of great importance in biocatalytic reactions is process temperature. It is widely known that this parameter directly affects both to reaction rate and to enzymes' activity and stability, and therefore, to process expenses. Furthermore, in solvent-free systems, increasing the temperature diminishes reaction mixture viscosity, and mass transfer and reaction rate are improved as a result (Serrano-Arnaldos et al., 2019). In addition, free CalB has an optimal temperature comprised between 50 and 60 ºC, but when immobilized in Novozym® 435, it can develop its maximum activity between 90 and 110 ºC, and remain active until 150 ºC (Murcia et al., 2020; Ragupathy et al., 2012).

Figure 4 represents the effect of temperature on the synthesis of the two diesters, where it can be concluded that, as expected, the formation rate of BEBA and BEBS and their intermediate products increase with temperature. As it can be seen, the maximum concentration values of intermediate MEBA and EEBS are mainly achieved after 30 min – 1 h of reaction depending on the assay, and during this time, the rate of formation of those intermediate esters is higher than the one for the final products. After this point, the transformation of the remaining DMA and DES into MEBA and EEBS happens more slowly than the synthesis of BEBA and BEBS from the intermediate species, even though, as it will be discussed below, the completion of this second alcoholysis reaction is not achieved.

In this figure, it can also be detected that the reactions carried out at 60 ºC stop with lower yields than those obtained at 50 ºC, causing "a crossing" between the curves corresponding to the highest and the lowest temperature. This anomaly happens when all the reagents are completely consumed (data not shown) and high concentrations of intermediate products are found in reaction mixture (15% of MEBA and 30% of EEBS at 60 ºC). As such fact might be explained by an evaporation of the EB, the experimental study of its vaporization at reaction temperature and stirring speed has been made, proving a loss of EB equal to 41% and 70% (w/w) after 6 hours at 50 and 60 ºC, respectively. These evidences confirm that the evaporation of EB is the main responsible for the unfinished synthesis of both diesters, as this decrease in the reactor content has a negative effect on the mass balance and reaction's equilibrium. Specifically, according to the residual amounts of intermediate products detected by the end of the temperature assays, concentrations between 5 and 9% (w/w) of EB may have

been lost by evaporation. These values are quite far that the ones observed during the evaporation test, as the most part of EB is transformed into products and the evaporation properties of this substrate may have been affected by the reaction mixture (the reaction rate might be higher than evaporation one).

In the light of the above results, 50 ºC was selected as the optimal temperature for the synthesis of BEBA y BEBS via lipase-catalyzed alcoholysis. Although losses of this substrate due to its vaporization would be minimized at lower temperatures, and several references regarding the synthesis of similar compounds at 40 ºC can be found in literature (Dormo et al., 2004; Hidalgo et al., 2018), experiments under 50 ºC have not been considered to avoid slowing down the reaction rate. On the contrary, different reaction strategies are proposed to accomplish this objective.

(FIGURE 4)

3.3. Reaction strategies

In order to reduce the release of EB into the surroundings, three different approaches involving diverse reactor configuration have been designed.

3.3.1. Fixed bed reactor

Reviewing the available bibliography, it can be found a successful example of obtaining adipic and sebacic acid esters in a packed bed reactor with different immobilized lipases, but isooctane was used as reaction media and the solvent-free synthesis was not considered (Ganguly and Nandi, 2015). In fact, even if "the best solvent is no solvent at all" (Guajardo and Domínguez de María, 2019), just a few studies deal with the solvent-free production of fatty acid esters in fixed bed reactors. Among them stand out those performed in a Tygon-like tube reactor with small diameter, where high yields where attained in the synthesis of esters from unsaturated fatty acids and glycerol catalyzed by an immobilized lipase from *Mucor miehei* (Arcos et al., 2000). Another interesting work explores the possibility of carrying out the biocatalytic production of biodiesel by using a *Burkholderia* sp. derivative in a series of packed bed reactors. In this case, the conversion achieved is relatively low (67%) (Tran et al., 2016).

In view of the foregoing, the synthesis of BEBA and BEBS was tried in a fixed bed reactor with the aim of reducing the available free surface of reaction mixture and, therefore, minimize EB evaporation (see *Diester synthesis in packed bed reactors* section). During these tests, flow through the packed bed was adversely affected, maybe by occlusion or blockage of the porous sintered glass plate, and such problem could not be mitigated by reducing recirculation flow rate from 0.09 mL s^{-1} to 0.04 mL s^{-1} after two hours. Besides, the difficulty to remove the byproducts generated during the

alcoholysis reaction (methanol or ethanol) derived in their accumulation in the reaction system and in the stop of the synthesis process because the chemical equilibrium was reached. These combined adverse effects led to final products with only a 20 – 30% (w/w) of diester, and thus, the fixed bed reactor strategy was discarded for the solvent-free synthesis of BEBA and BEBS.

3.3.2. Fed batch reactor

A fed batch tank reactor with stepwise additions of EB as specified in Table 1 was also studied in order to reduce the amount released to the atmosphere. First of all, the synthesis was performed by adding five batches of the equal amounts of EB at constant time intervals of one hour. As it can be noticed in Figure 5, during the first two hours EB concentration was too small and that caused the reaction to be paused for a certain time interval before the next substrate addition, after which it was restarted again. As a consequence, after 5 hours, final concentration on BEBA and BEBS was similar for both reaction systems and close to 75% (w/w), while significant amounts of substrates and intermediate products were also detected.

(FIGURE 5)

In order to increase reaction yield, the stepwise addition pattern was slightly changed by performing the first two additions at 0 and 30 min, maintaining the remaining EB batches at intervals of an hour. Despite this modification, results were barely improved (data not shown), which is in contrast with the yields of 95% achieved by the authors during the stepwise synthesis of neopentyl glycol diheptanoate (Serrano-Arnaldos et al., 2020). Therefore, the fed batch reactor strategy was neither considered for further tests.

3.3.3. Batch reactor with excess of EB

An alternative method for the enzymatic synthesis of BEBA and BEBS consisting in operating with an excess of EB was also tried. This approach has been successfully used in similar solvent-free systems with the purpose of increasing reaction rate (Kim et al., 2019; Lee et al., 2019) and, in this particular case, it will also compensate the losses of EB by evaporation. In order not to increment process cost and to keep as high as possible the percentage of carbon in the reagents that remains in the final product (quantified by the Carbon Mass Efficiency, CME) (Lima-Ramos et al., 2014), two assays were carried out with small excesses of EB calculated based on the results of the experimental study of its vaporization described in *Influence of biocatalyst concentration and temperature* section. So, a 15% and a 25% molar excess of EB were tried, which correspond to molar ratio (DMA:EB or DES:EB) of 1:2.3 and 1:2.5,

respectively. Best results were reached with a 15% of excess for the synthesis of BEBA (Figure 6 A), while for BEBS with a molar excess of 25% (Figure 6 B), making patent the negative influence of acid chain length in the reaction. Such excesses of EB are relatively low compared with those used by other researchers in similar reaction systems: 1:4 (Gryglewicz, 2001; Kim et al., 2019), 1:5.3 (Chaibakhsh et al., 2009) or even 1:7.7 (Rahman et al., 2011), molar ratios that are quite far from stoichiometric ones.

As a result of this reaction strategy, products with a final content on diester close to 95% (w/w) were obtained after 5 hours of synthesis. To the best of our knowledge, this is the first time that the enzymatic production of BEBA and BEBS is described, and that is why they can only be compared with the available literature for other biosynthesized compounds, such as diethylhexyl adipate, whose esterification process was studied by Kim et al. (2019). In this work, the synthesis was performed under vacuum conditions, which contributes to the shift of the chemical equilibrium towards ester formation (and hence, shortens reaction time), as 2-ethylhexanol is practically a non-volatile liquid at room temperature (Narayan and Madras, 2017).

(FIGURE 6)

3.4. Biocatalyst reuse

The possibility of reusing the biocatalysts is one of the major factors that contribute the viability of an industrial bioprocess, as it can substantially reduce its final cost. Consequently, the ability of Novozym® 435 in retaining its activity after several uses has been tested by performing seven consecutive reactions of 5 hours under optimum conditions (Figure 7). After each use, the immobilized derivative was recovered from reaction medium, washed with acetone and air-dried at room temperature, in a way that losses of biocatalyst between the first and the last assay were lower than 12% for both reaction systems. From Figure 7, it can be concluded that Novozym® 435 can be reused at least seven times for obtaining both BEBA and BEBS without an apparent loss of its activity, as final product concentration was the almost the same at the end of the reaction cycle. In this sense, it must be pointed out that the good reusability of Novozym® 435 might be rather attributed to stirring system used during the reusability test (two bladed vertical stirrer) than to the mechanic stability of its carrier, as the nefarious effect of other agitation devices, such as magnetic stirrers, has already been described in the bibliography (Ortiz et al., 2019).

Besides, in what regards BEBS (Figure 7 B), conversions obtained after one hour of use reflect again, the negative effect of the length of the dicarboxylic acid chain on reaction evolution. Despite this fact, it should be noted that final product concentration in BEBS

above 90% after the biocatalyst reuses, approximately 3-4 percentage units less than BEBA's final concentration depending on the assay.

In view of the above mentioned, it can be affirmed that Novozym® 435 can be successfully recovered and reused at least seven times for the solvent-free production of BEBA and BEBS.

(FIGURE 7)

3.5. Green, process and economic metrics of the biocatalytic synthesis

In order to emphasize the benefits of this enzymatic process and to pre-evaluate the viability of its possible industrial implementation, some of the most important indicators regarding the sustainability, productivity and economy of the process have been calculated (Sheldon and Woodley, 2018; Sheldon, 2017, Lima-Ramos et al., 2014; Murcia et al., 2020). For that purpose, data from the reactions carried out under optimal conditions have been used, and the results obtained are shown in Table 2.

Table 2. Green, process and economic metrics for the biocatalytic synthesis of BEBA and BEBS.

Scaling-up prospective		**BEBA**	**BEBS**
Green metrics	Atom economy (AE)* (%)	83.07	77.88
	E-factor**	0.32	0.46
	Carbon mass efficiency (CME)*** (%)	72.79	60.25
Process metrics (productivity)	(kg product)×(kg biocatalyst)$^{-1}$	37.88	38.16
	(kg product)×(L of reactor)$^{-1}$×h^{-1}	0.165	0.166
Economic metrics €×(kg product)$^{-1}$	Substrates cost	224.48	264.28
	Biocatalyst cost	34.32	34.07
	Energy cost	0.55	0.55
	Total cost	259.35	298.90

$$** \ AE = \frac{\text{molecular weight of desired product}}{\sum \text{molecular weight of all products}} \times 100 = \frac{\text{molecular weight of desired product}}{\sum \text{molecular weight of all reactants}}$$

$$** \ E-\text{factor} = \frac{\text{kg waste}}{\text{kg of desired product}}$$

$$*** \ CME = \frac{\text{kg carbonated product}}{\text{kg carbonated reactants}} \times 100$$

The selected green metrics in Table 2 are the most widely used as they allow to easily quantify the sustainability of all synthetic processes, including biocatalysis. As can be seen, the atom economy (AE) values are quite close to 100% for BEBA and BEBS, which indicates the sustainability of both biocatalytic systems despite that carbonated by-products are released (carbon mass efficiency, CME). Furthermore, the E-factor quantifies the waste generated during a process and as it can be seen, the calculated value for both processes is small and clearly below the usual values calculated in the manufacture of fine chemicals (5 – 50) (Sheldon, 2017). It should be noted that these calculations have taken into account not only the masses of methanol and ethanol released during the enzymatic reactions, but also the excess of EB used to compensate the one vaporized into the atmosphere. Comparing the two processes, it is clear that the enzymatic synthesis of BEBS is less sustainable than the one of BEBA because of the higher molecular weight of the alcohol released into the atmosphere (methanol vs. ethanol).

Additionally, two of the most used indicators of biocatalytic processes' productivity have been calculated, since they are a reflection of the real possibilities that the enzymatic pathway is taken to an industrial scale. As it can be observed in Table 2, the calculated values for these process metrics are quite high, which is due to the fact that a solvent-free reaction medium has been used (and thus, the content of the reactor is mainly composed of substrates and products) and that conversions of ~95% are achieved in only 5 hours by using low concentration of Novozym® 435.

Finally, a preliminary cost estimation has been performed (Murcia et al., 2020; Serrano-Arnaldos et al., 2019), pointing out that producing BEBA and BEBS at laboratory scale is relatively expensive (Table 2), as the contribution of the substrates to the final price is more than 85% of the total. On the other hand, the influence of the cost of the immobilized enzyme is lower than 14% for both products. Nevertheless, these total cost values are higher than those recommended in the literature (Tufvesson et al., 2011), although they could be reduced if bulk substrates were used and the biocatalyst is reused at least up to seven reaction cycles, according to the reusability test.

In any case, the metrics gathered in Table 2 support the future viability of the proposed biocatalytic processes and the need to perform the appropriate scaling-up studies in order to achieve their industrial implementation.

4. CONCLUSIONS

The ability of the immobilized CalB lipase, marketed as Novozym® 435, to catalyze the synthesis of the ethyl butyl diesters of adipic and sebacic acids has been demonstrated for the first time. The high volatility of the alcohol used forces to perform the reaction

with an excess of the same in order to compensate substrate losses and achieve high products yields. During the study of different reaction strategies, the process has been optimized in a batch reactor: 50 ºC, 2.5% (w/w) of biocatalyst and a 15% of molar excess of alcohol for the adipic diester and 25% for the sebacic one. Even though a negative impact of the acid chain length has been observed, under best reaction conditions, bis(2-ethylbutyl) adipate and bis(2-ethylbutyl) sebacate concentration in the final product was similar. Thus, this work demonstrates that the enzymatic synthesis of branched diesters with lubricant applications is easy to perform and low energy consuming, which results in an environmentally-friendly process potentially viable at industrial scale.

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DECLARATION OF INTEREST

Declarations of interest: none.

SUPPLEMENTARY MATERIAL

^{13}C NMR of biosynthesised BEBA (Figure S1) and BEBS (Figure S2).

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FIGURE CAPTIONS

Figure 1. Reaction scheme of the enzymatic synthesis of BEBA and BEBS.

Figure 2. Preliminary experiments in the synthesis of BEBA (A: ○ EB, ▼ DMA, □ MEBA, ♦ BEBA) and BEBS (B: ○ EB, ▼ DES, □ EEBS, ♦ BEBS) with Novozym® 435 (batch reactor, 20 g, 1:2 molar ratio, 2.5% (w/w) of biocatalyst and 60 ºC).

Figure 3. Influence of biocatalyst concentration in the synthesis of BEBA (A) and BEBS (B): ● 1.25%, ▼ 2.5% and ■ 5% of Novozym® 435 (batch reactor, 20 g, 1:2 molar ratio and 60 ºC).

Figure 4. Influence of temperature in the synthesis of BEBA (A) and BEBS (B): ● 50 ºC, ○ 60 ºC, ····· MEBA or EEBS, —— BEBA or BEBS (batch reactor, 20 g, 1:2 molar ratio and 2.5% (w/w) of biocatalyst).

Figure 5. Synthesis of BEBA (A: ○ EB, ▼ DMA, □ MEBA, ♦ BEBA) and BEBS (B: ○ EB, ▼ DES, □ EEBS, ♦ BEBS) in a fed batch reactor (20 g, 1:2 molar ratio, 5 EB additions at intervals of 1 hour, 2.5% (w/w) of biocatalyst and 50 ºC).

Figure 6. Synthesis of BEBA (A: ○ EB, ▼ DMA, □ MEBA, ♦ BEBA) with a 15% molar excess of EB and BEBS (B: ○ EB, ▼ DES, □ EEBS, ♦ BEBS) with a 25% molar excess of EB (batch reactor, 20 g, 2.5% (w/w) of biocatalyst and 50 ºC).

Figure 7. Reusability test of Novozym® 435 under best operation conditions for the synthesis of BEBA (A) and BEBS (B): black bars – 1 h of reaction, grey bars – 5 h of reaction (batch reactor, 20 g, 2.5% (w/w) of biocatalyst, 50 ºC, 15% EB excess for BEBA and 25% EB excess for BEBS).

SUPPLEMENTARY MATERIAL CAPTIONS

Figure S1. ^{13}C NMR spectrum of bis(2-ethylbutyl) adipate ($CDCl_3$, 400 MHz). δC: 173.4 C5; 66.2 C4; 40.1 C3; 33.8 C2; 24.2 C6 and C7; 10.8 C1.

Figure S2. ^{13}C NMR spectrum of bis(2-ethylbutyl) sebacate ($CDCl_3$, 600 MHz). δC: 173.9 C5; 66.2 C4; 40.3 C3; 24.8 – 34.33 C2, C6, C7, C8, C9; 11.0 C1. One of the C6 to C9 carbons does not appear because of the similarity of the chemical environment of those atoms.

Figure 1

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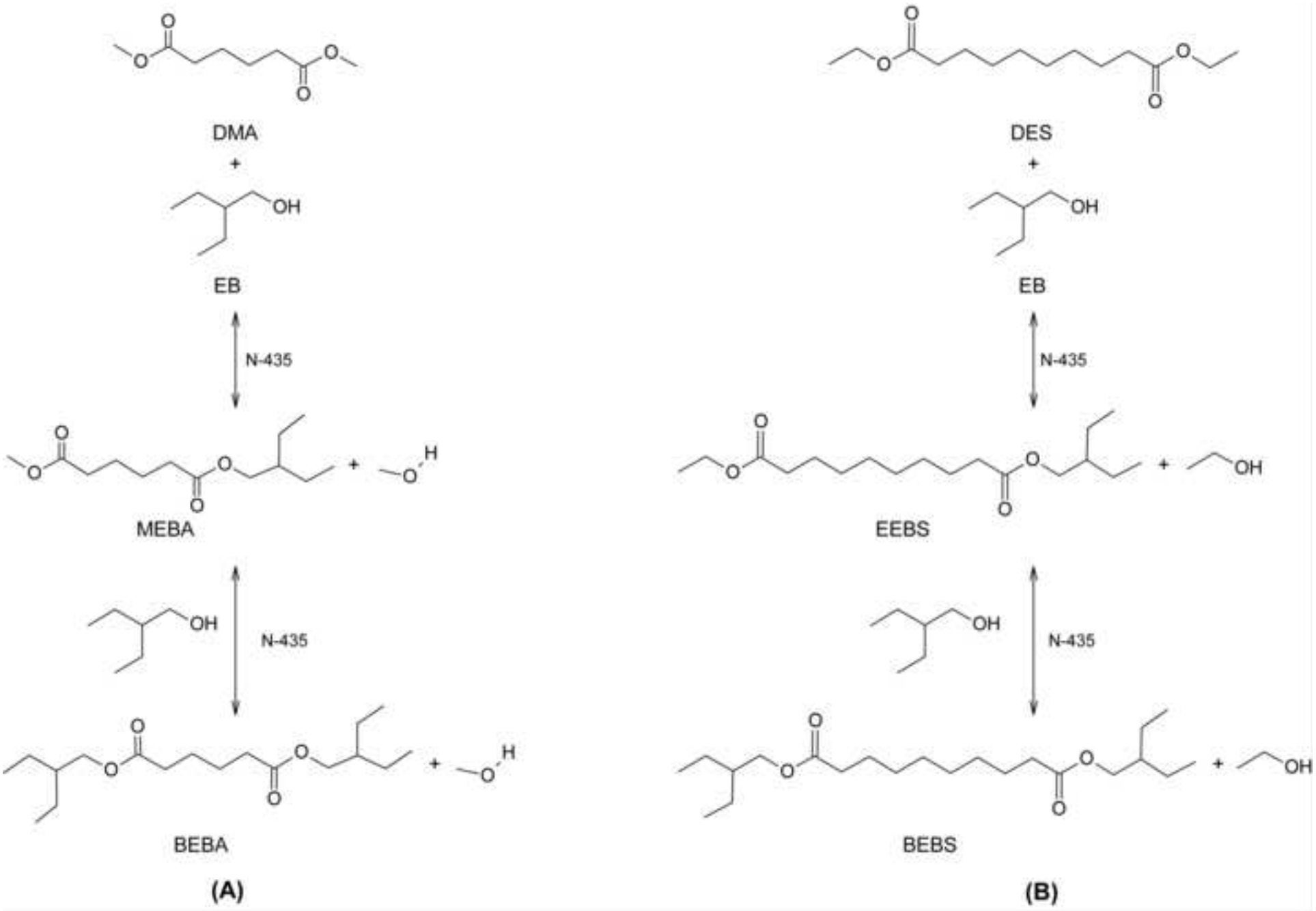

Figure 2

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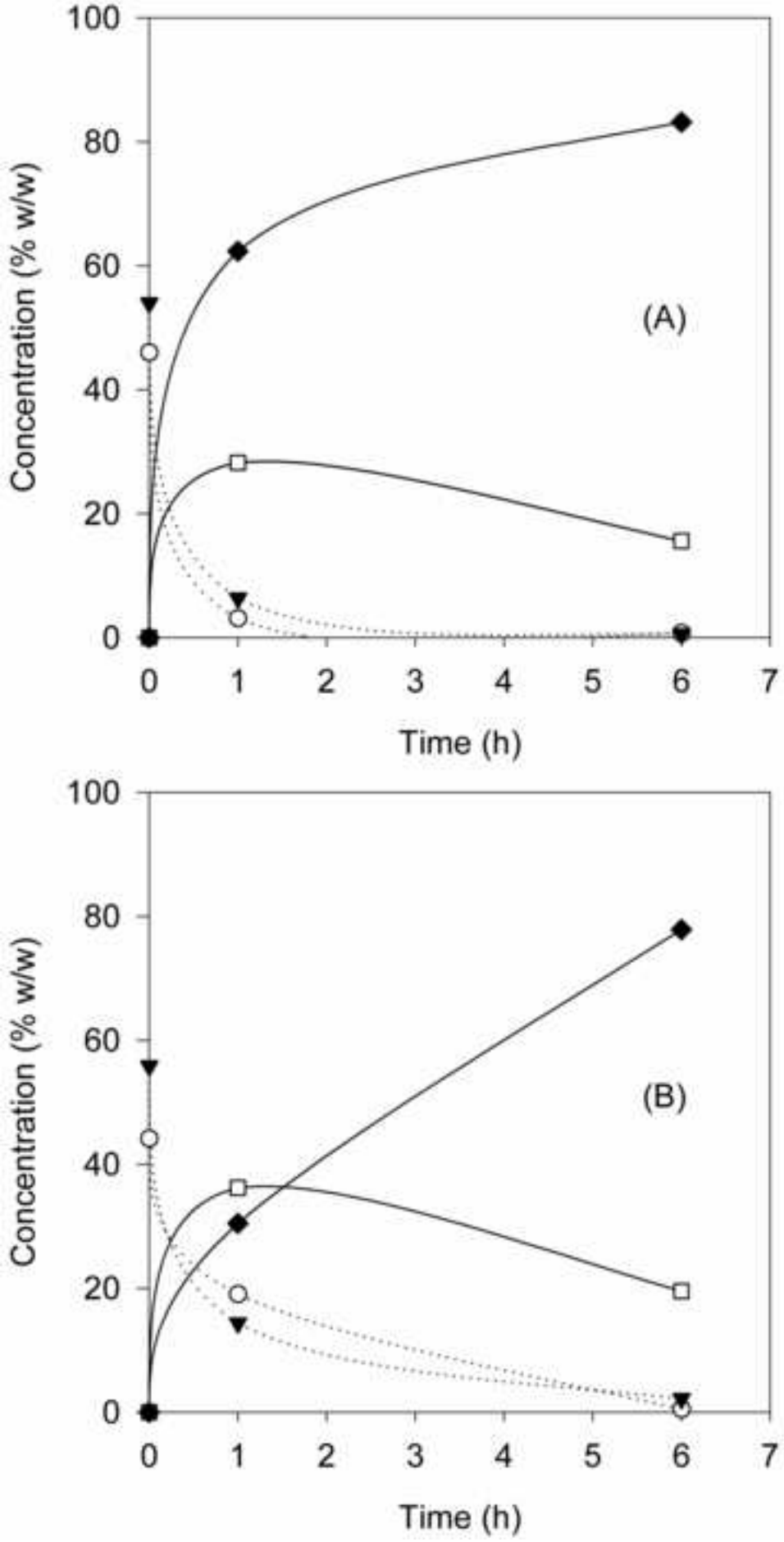

Figure 3

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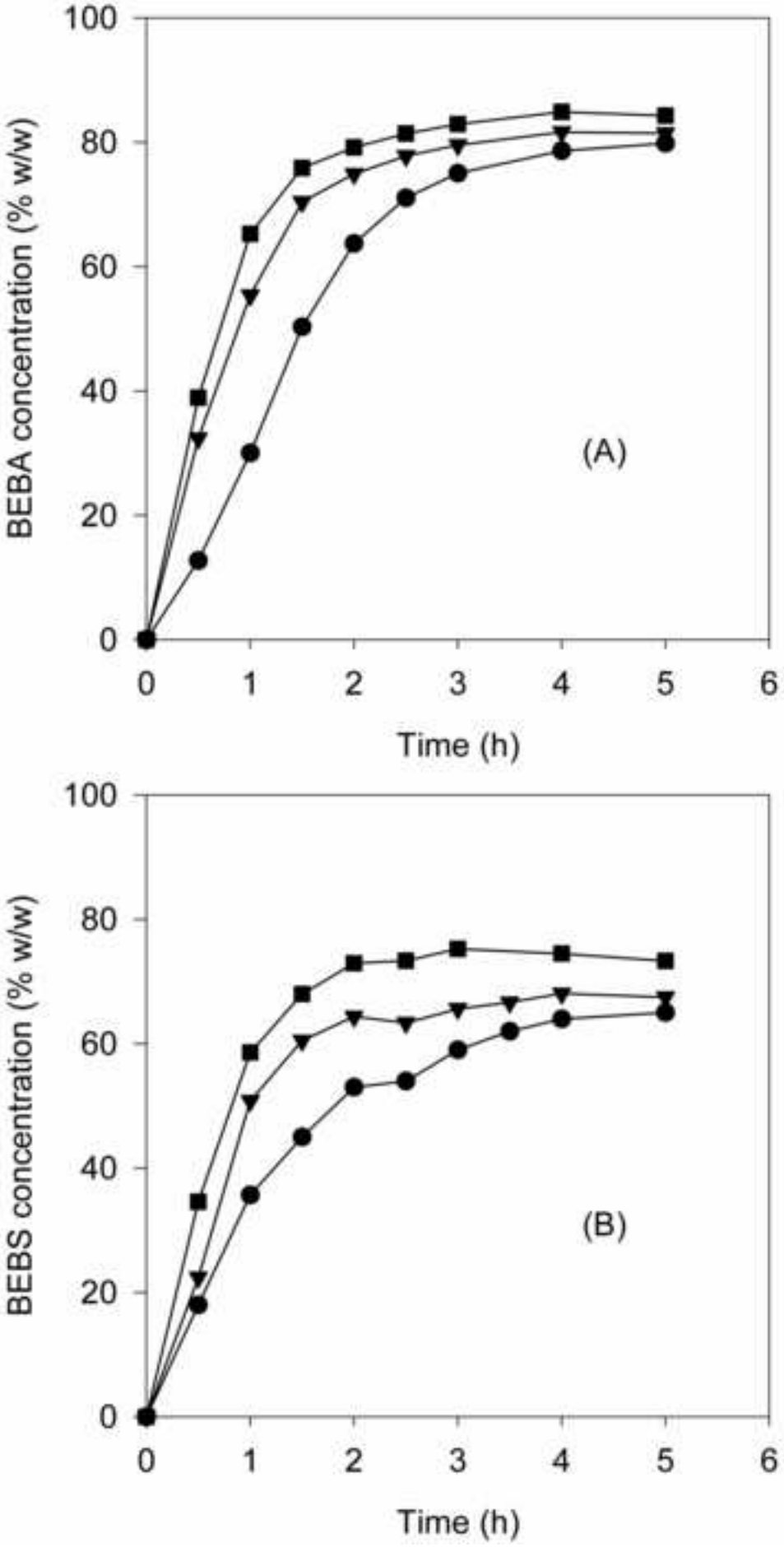

Figure 4

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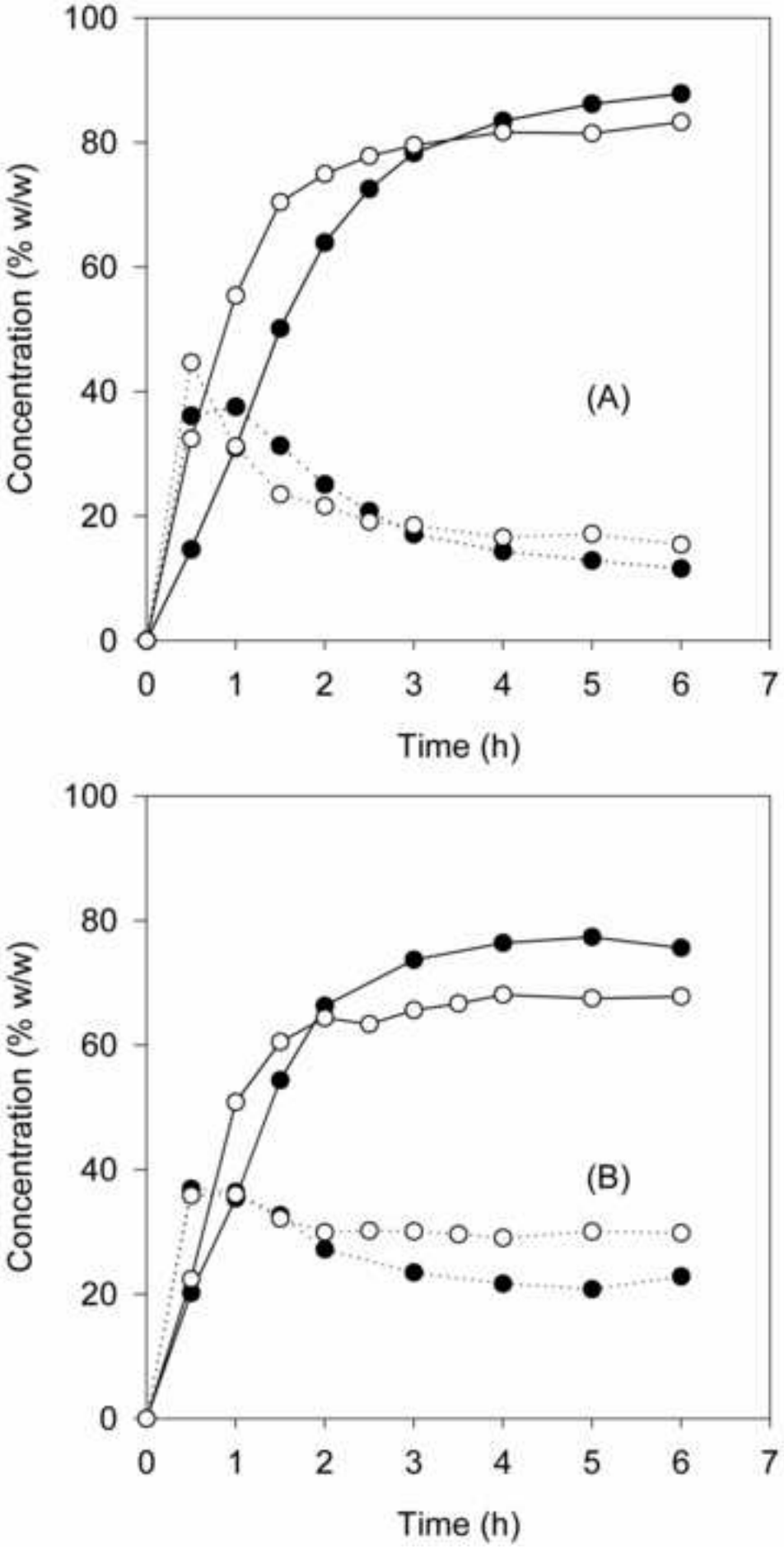

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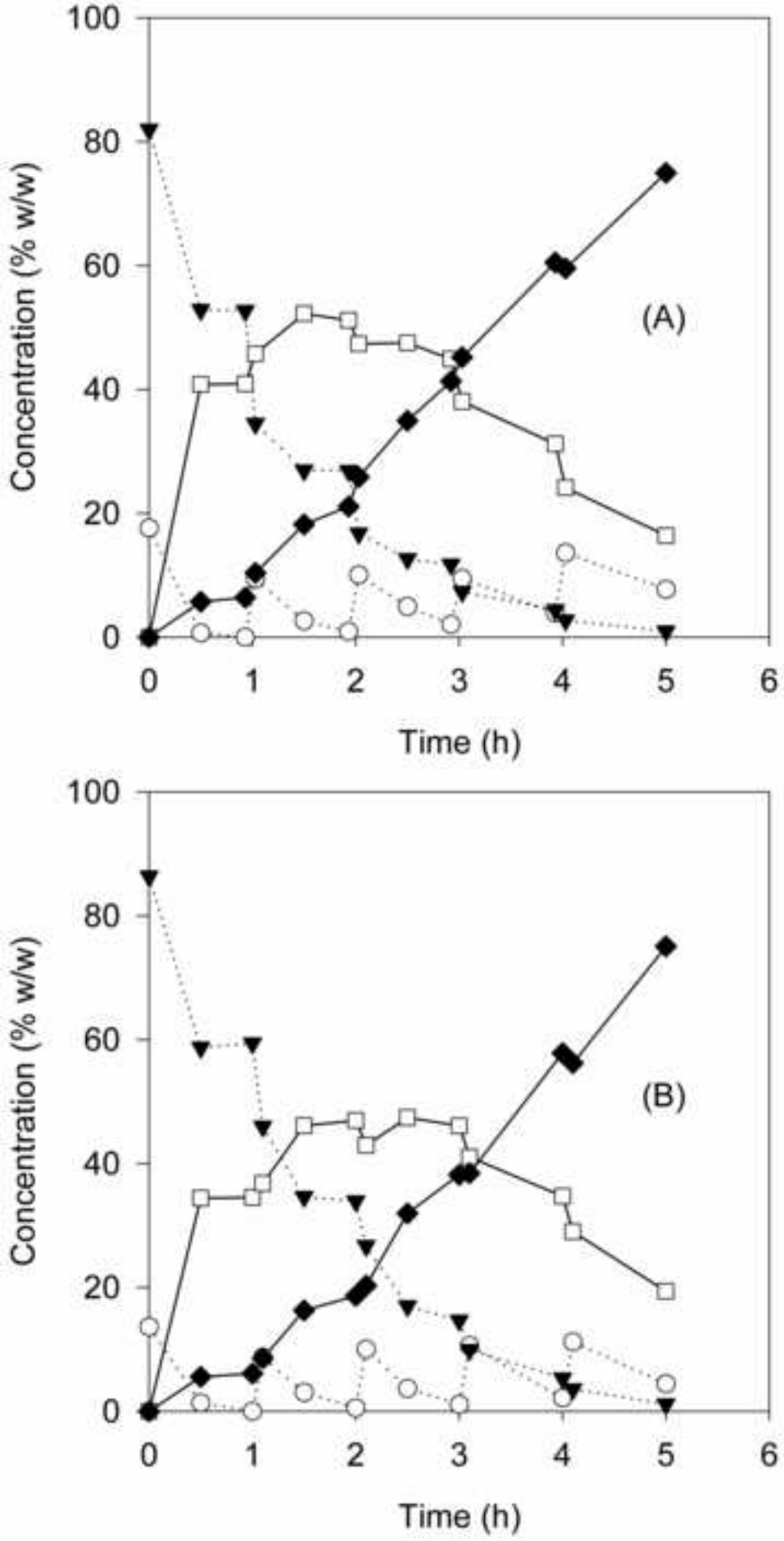

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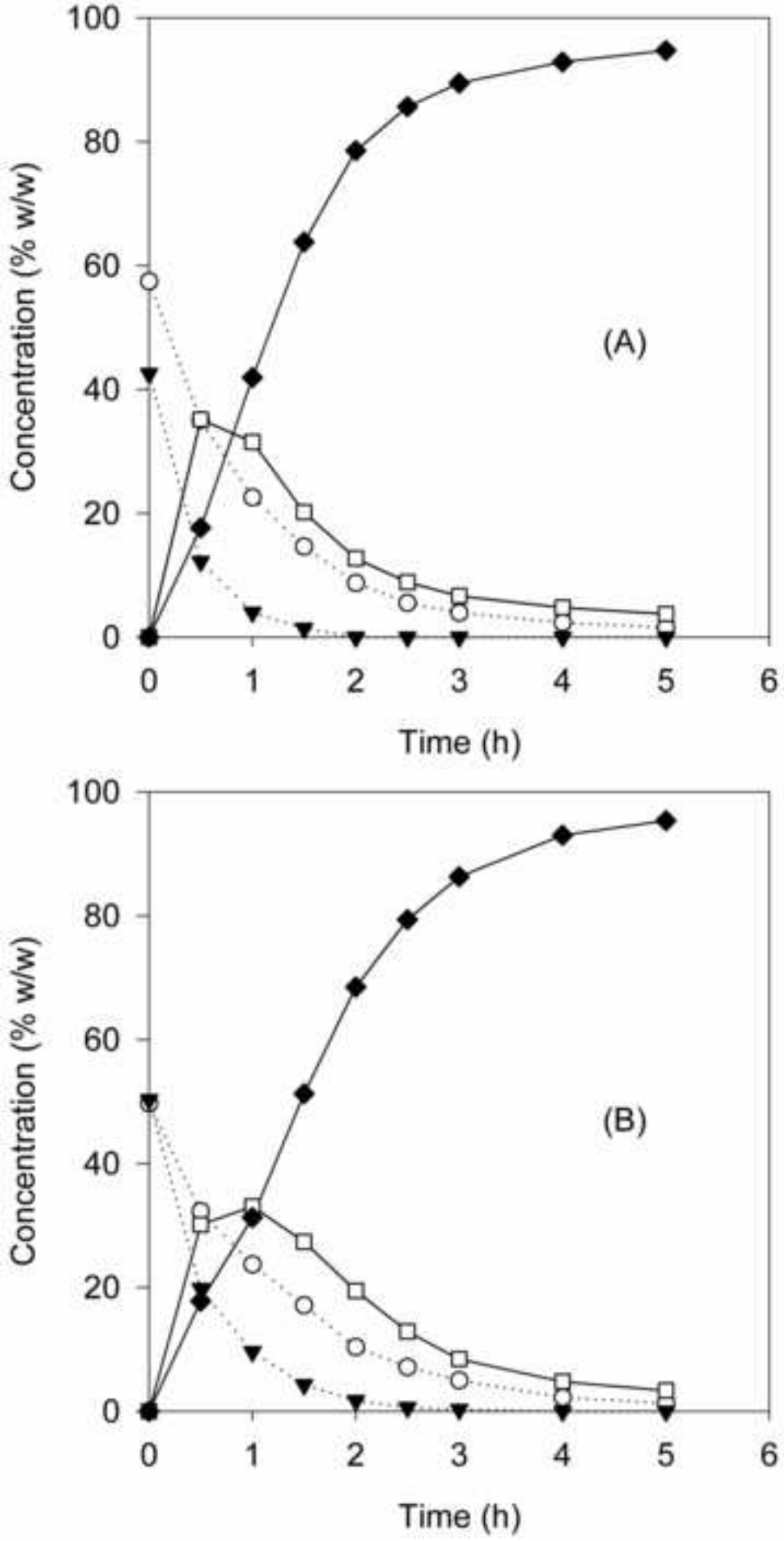

Figure 7 review

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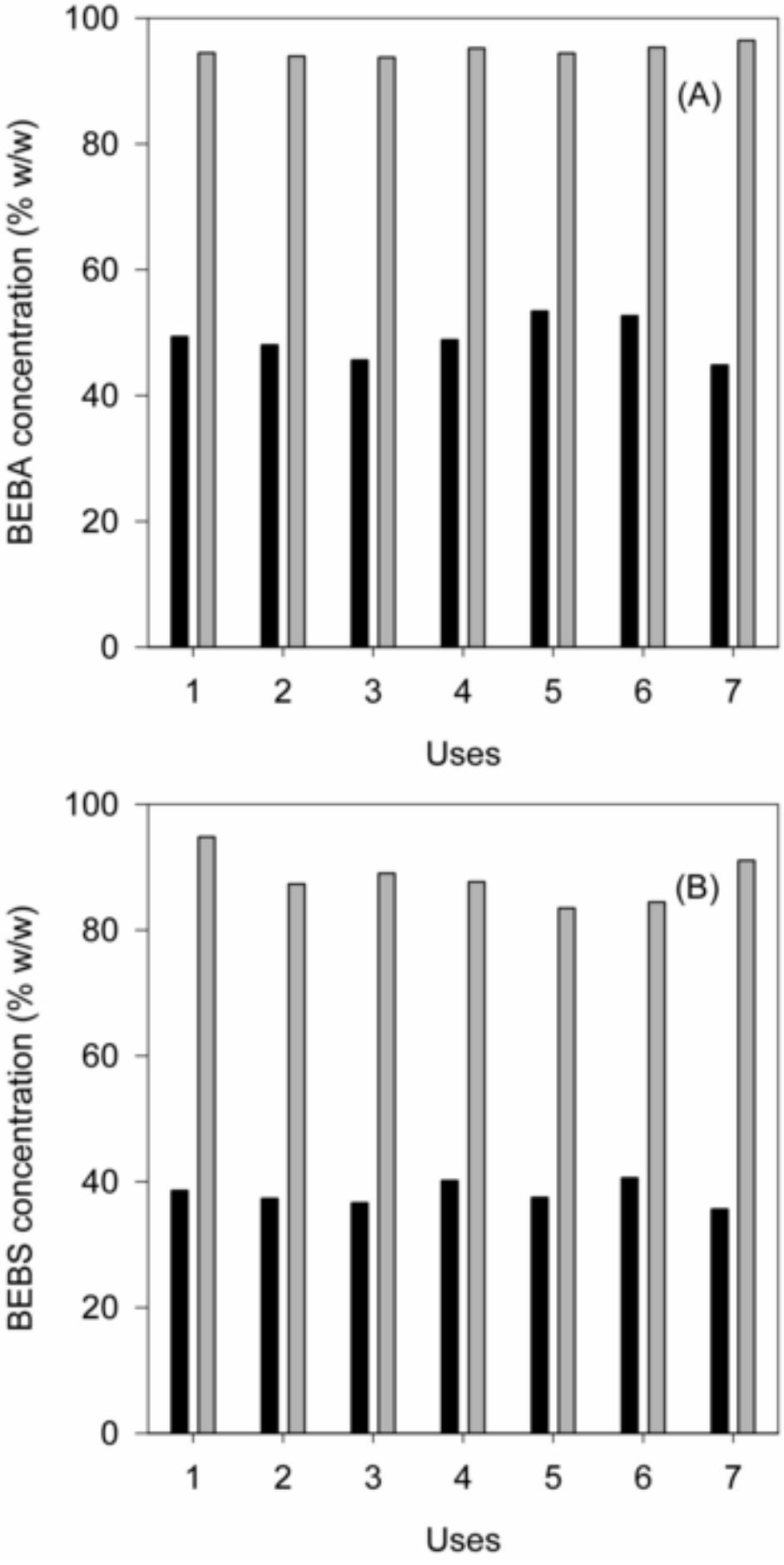

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