Vesna Kučan Polak

ISSN 0350-350X
GOMABN 45, 4, 217-228
Stručni rad/Professional Paper
UDK 665.3.095.13 : 66.098 : 577.153 : 665.3.094.942 : 66.095.13

PROIZVODNJA BIODIZELSKOGA GORIVA ENZIMSKOM TRANSESTERIFIKACIJOM

Sažetak

Zanimanje i ideja o korištenju biljnih ulja kao zamjene za mineralna ulja i proces transesterifikacije stari su više od stotinu godina. Danas se u industrijskoj proizvodnji biodizelskoga goriva koristi nekoliko procesa transesterifikacije kojima se ostvaruju visoke konverzije triglicerida u odgovarajući metilni ester. U zadnje se vrijeme intezivno istražuje enzimski katalizirana transesterifikacija koja zbog uočenih prednosti sve više naglašava potencijal ovog procesa za proizvodnju biodizelskoga goriva.

Uvod

U globalnoj i potrošačkoj ekonomiji sve se više povećavaju ljudske potrebe i sve su zahtjevniji uvjeti koji se postavljaju pred kemijske industrijske procese. Ograničenja najvažnijih energetskih resursa i prihvaćanje Kyoto protokola (1999. god.) nameću obveze koje zahtijevaju nove modalitete za primjenu tehnologija pogodnih za okoliš, sigurnu proizvodnju s obzirom na zdravlje i sigurnost ljudi, proizvodne procese sa što manjim utroškom sirovina i energije, s mogućnošću recikliranja i sl.

Iako danas udio obnovljivih izvora energije u ukupnoj potrošnji u EU iznosi oko 6%, u budućnosti se predviđa značajan porast, te se XXI. stoljeće naziva i stoljećem obnovljive energije. (1)

Od svih obnovljivih izvora energije najviše mogućnosti pruža biomasa, a biogoriva proizvedena iz biomase osim zadovoljenja EU Direktiva koje propisuju uvođenje takvih goriva zauzimaju i sve važnije mjesto u nacionalnoj ekonomiji pojedinih država EU. (2)

Danas se u industrijskoj proizvodnji biodizelskoga goriva koriste različite tehnologije, a najviše je razvijen način proizvodnje procesom transesterifikacije alkalnim katalizatorom kojim se ostvaruju visoke konverzije masti odnosno ulja u odgovarajuće estere u kratkom vremenu. Budući da ekonomičnost ovih procesa ovisi o nekoliko bitnih čimbenika, npr. kvaliteti sirovine, tj. sastavu sirovine, potrošnji energije, odvajanju i čišćenju nusproizvoda, obradi otpadnih voda i sl., ispituju se razne mogućnosti poboljšanja i pomoću biokatalitičkih konverzija, tj. enzimske tehnologije.

Enzimska transesterifikacija

Enzimska tehnologija je interdisciplinarno područje, koje je OECD (Organization of Economic Cooperation and Development) prepoznao kao važnu komponentu održivog industrijskog razvoja. Enzimi koji se koriste u reakcijama s masnim kiselinama, mono- i diacilglicerolima i glicerolom – lipaze - igraju ključnu ulogu u razgradnji lipida. (3, 4) Zadnjih desetak godina povećano je zanimanje u istraživanju lipaza zbog tri osnovna razloga:

1. enzimska paradigma, tj. katalitička funkcija lipaza: lipaze iako topljive u vodi kataliziraju reakcije koje uključuju netopljive lipidne supstrate na međufaznoj površini lipid-voda, što je karakteristika jedinstvene strukture lipaza,
2. važnost lipaza u medicini, tj. njihova važnost u regulaciji metabolizma,
3. otkriće lipaza kao alata za kataliziranje ne samo hidrolize nego i različitih reverzibilnih reakcija, kao esterifikacija, transesterifikacija i aminoliza u organskim otapalima.

Biokatalizatori imaju određene prednosti u odnosu na klasične katalizatore, a to su njihova specifičnost, regioselektivnost i enantioselektivnost koje omogućavaju katalitičke reakcije sa smanjenim nusproduktima, manjim troškovima obrade otpada i uvjetima reakcija koji su blaži glede temperature i tlaka.

Tablica 1: Industrijska primjena lipaza (5)

Područje	Primjena	Proizvod
hidroliza		
hrana	hidroliza mlijeka (mliječne masti)	zaslađivači za mliječne proizvode
Kemijska proizvodnja masnoća	hidroliza ulja i masti	masne kiseline, digliceridi i monogliceridi, reagenti za analizu lipida
Kemijska proizvodnja površinsko aktivnih tvari	analiza distribucije masnih kiselina u trigliceridima, uklanjanje uljnih mrlja, kapljica i lipida	detergenti za rublje i kućanstvo
medicina	krvna analiza triglicerida	dijagnostički alat
esterifikacija		
Kemijska proizvodnja - specijalne kemikalije	sinteza estera	kiralni intermedijari, esteri, emulgatori
hrana-kemijska i farmaceutska proizvodnja	transesterifikacija prirodnih ulja	ulja i masti

Zbog svojstva očuvanja katalitičke aktivnosti u organskim otapalima, ispitivane su aktivnosti lipaza kao katalizatora kako bi se odredio njihov potencijal za konverziju viška masti i ulja u visokovrijedne proizvode za hranu i industrijsku uporabu, a daljnji su primjeri primjene nađeni u rezoluciji racemičnih smjesa, sintezi farmaceutskih proizvoda i surfaktanata, biokonverziji ulja i masti.

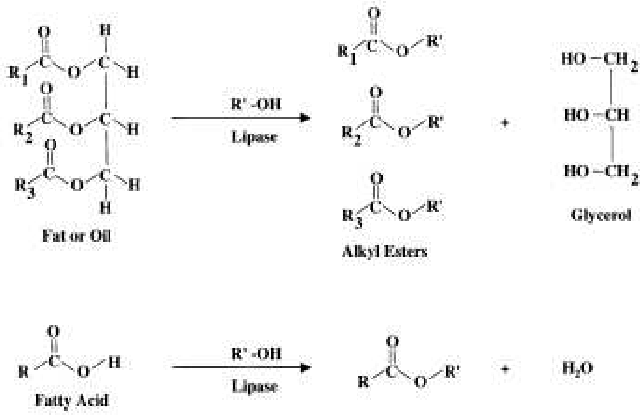
Biološka funkcija lipaza je kataliza hidrolize estera, posebno dugolančanih triacilglicerola, kao i reverznih reakcija; esterifikacije, transesterifikacije, aminoliza, oksimoliza i tiotransesterifikacija u anhidridnim otapalima, bifaznim sustavima i micelarnim otopinama. Primjeri industrijske primjene lipaza navedeni su u tablici 1.

Kako bi korištenje lipaza bilo ne samo učinkovito, nego i ekonomski isplativo u vodenim i nevodenim otapalima, povećana su i istraživanja tehnika njihove modifikacije koje se mogu svrstati u tri kategorije: kemijska modifikacija, fizikalna modifikacija i genetički inženjering, a izvode se kako bi se povećala aktivnost, selektivnost ili stabilnost i poboljšanje topljivosti u organskim otapalima.

Fizikalna modifikacija koja se najviše primjenjuje na lipazama je imobilizacija, proces koji povezuje enzim na netopljivu krutu podlogu, a metode se mogu klasificirati u 3 grupe: adsorpcija na noseći materijal, uklapanje i kapsulacija i kovalentno spajanje na aktivnu matricu.

Uporaba biokatalizatora u transformaciji masti/ulja kao i glicerida i masnih kiselina u visokovrijedne proizvode dokumentirana je brojnim radovima, čiji je cilj bio učinkovito korištenje imobiliziranih lipaza kao katalizatora pri pridobivanju jednostavnih estera biljnih ulja. (4, 5)

Slika 2: Reakcija esterifikacije s lipazama



Proizvodnja biodizela iz nerafiniranih sirovina kao što su rabljena ulja i masti donosi poteškoće koje su rezultat svojstava takvih sirovina i /ili sastava slobodnih masnih kiselina. Stoga se i krenulo u smjeru poboljšanja procesa proizvodnje kao što su istraživanja enzimske alkoholize biljnih i životinjskih ulja/masti u otapalima i sustavima bez otapala, korištenjem primarnih i sekundarnih alkohola. Korištenje imobiliziranih lipaza u industrijskoj primjeni vrlo je značajno, naročito od kada se imobilizirane lipaze mogu višestruko upotrebljavati što bitno umanjuje troškove katalizatora.

Pri korištenju rabljenih jestivih ulja iz ugostiteljstva i masti kao sirovina za dobivanje alkilnih estera, dobiveni su prinosi veći od 95% korištenjem različitih komercijalnih lipaza. Korišten je enzim IM ps-30 koji ima potencijal za proizvodnju biodizela, s prinosom od 98% estera iz korištenih masnoća iz ugostiteljstva, a raniji radovi s ovim enzimom pokazali su da se može koristiti i više puta. Na slici 2 prikazan je primjer reakcije esterifikacije s lipazama kao katalizatorima.

U procesima esterifikacije sa lipazama kao katalizatorima istraživani su različiti tipovi alkohola - primarni, sekundarni, ravnolančani i razgranati, a rezultati takvih procesa tj. postignuta konverzija prikazana je u tablici 2. Kratkolančani alkoholi (metanol, etanol) mijenjaju svojstva lipaza što se može negativno odraziti na reakciju, pa se ispituju novi načini proizvodnje biodizela korištenjem metil-acetata kao acil-akceptora koji uz lipazu Novozym 435 (C. antarctica) daje iskorištenje od 92 %. (6,7)

Tablica 2: Primjeri konverzija nekih enzima kao katalizatora u reakcijama transesterifikacije (8)

Ulje	Alkohol	Lipaza	Konverzija%	Otapalo
Uljana repica	2-etil-1-heksanol	*Candida rugosa*	97	ne
Mango	C_4-$C_{18:1}$-alkoholi	*Mucor miehei* (Lipozyme IM-20)	86,8-99,2	ne
Suncokret	etanol	*Mucor miehei* (Lipozyme)	83	ne
Riba	etanol	*Candida antarctica*	100	ne
Ulja iz restorana	etanol	*Pseudomonas cepacia* (Lipase PS 30) *Candida antarctica* (Lipase SP 435)	85.4	ne
Loj, soja, uljana repica	prim. alkohol.* sek. alkohol,** metanol, etanol	*Mucor miehei* (Lipozyme IM60) *Candida antarctica (SP435)* *Mucor miehei* (Lipozyme IM60) *Mucor miehei* (Lipozyme IM60)	94,8-98,5 61,2-83,8 19,4 65,5	heksan heksan ne ne
Suncokret	metanol metanol etanol	*Pseudomonas fluorescens*	3 79 82	ne petrol eter ne
Sjemenke palme	metanol etanol	*Pseudomonascepacia* (Lipase PS 30)	15 72	ne ne

*metanol, etanol, propanol, butanol, izobutanol
**izopropanol, 2-butanol

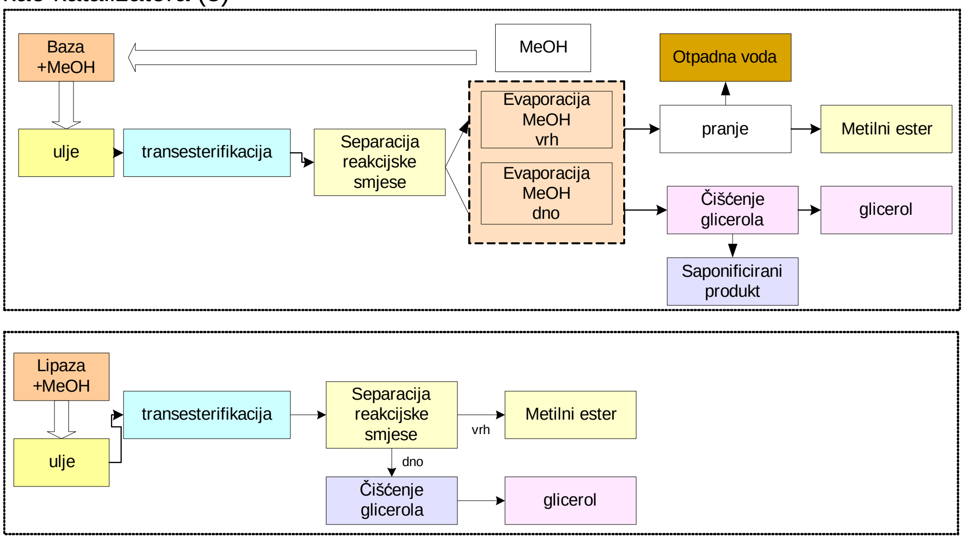
Usporedba između alkalno katalizirane reakcije i lipazama katalizirane reakcije za proizvodnju biodizela dana je u tablici 3.

Tablica 3: Usporedba alkalno katalizirane reakcije i lipazama katalizirane reakcije za proizvodnju biodizelskog goriva (8)

	Alkalno katali irani proces	Proces s lipazama kac katalizatorom
Reakcijska temperatura	60º-70ºC	30º-40ºC
Sl. masne kiseline u sirovini (FFA)	sapuni	metilni ester
Voda u sirovini	sprječava reakciju	nema utjecaj
Prinos metilnog estera	visok	visok
Pridobivanje glicerola	teško	lagano
Čišćenje metilnog estera	višestruko pranje	ne
Proizvodnja katalizatora	nije skupa	relativno skupa

Danas se u komercijalnoj proizvodnji biodizela najviše koristi alkalno katalizirani proces, a poteškoće koje nastaju u procesu najčešće su vezane za uklanjanje katalizatora iz otpadnih voda, evaporaciju metanola, čišćenje glicerola, uklanjanje saponificiranih proizvoda, dok u procesima s lipazama kao katalizatorima one nisu prisutne. Slika 3 prikazuje usporedbu proizvodnje biodizelskog goriva pomoću baznog katalizatora i lipaza kao katalizatora.

Slika 3: Dijagram toka proizvodnje biodizela uporabom alkalnog katalizatora i lipaza kao katalizatora (8)



Zbog navedenih prednosti treba pretpostaviti da će daljnja istraživanja lipaza kao katalizatora u proizvodnji biodizelskog goriva i dalje biti intezivna. Korištenje, kao i opseg različite biotehnološke primjene lipaza je u porastu, a konstantna optimizacija lipaza i reakcijskih uvjeta sve više omogućava i ekonomičnu proizvodnju visokovrijednih kemikalija i farmaceutskih proizvoda.

Zaključak

Biodizelsko gorivo postaje sve atraktivnije gorivo zbog svojih ekoloških značajki i činjenice da predstavlja obnovljivi izvor energije.

Specifičnost reakcija kataliziranih enzimima - lipazama rezultira korištenjem tih enzima u mnogim procesima sinteze ili modifikacija masti ili ulja. U usporedbi s konvencionalnim kemijskim procesima, enzimski katalizatori uspješno kataliziraju reakcije pri mnogo blažim uvjetima temperature i tlaka, a poteškoće u postojećim procesima proizvodnje biodizelskog goriva najčešće vezane za uklanjanje katalizatora iz otpadnih voda, evaporaciju metanola, čišćenje glicerola, uklanjanje saponificiranih proizvoda u procesima s lipazama kao katalizatorima nisu prisutne.

Istraživanja biokatalizatora u reakcijama esterifikacije, odnosno uporaba enzima kao katalizatora, na mikrorazini povezuje područje biotehnologije i kemijskog inženjerstva, koje bi moglo nastaviti razvoj i dimenzioniranje ovih procesa u većim mjerilima kada se riješi pitanje cijene ove vrste katalizatora koja je još uvijek previsoka za industrijsku uporabu.

Biotehnologija i genetički inženjering zasigurno će odigrati važnu ulogu u proizvodnji lipaza za industrijsku primjenu, a budućnost će pokazati da li će u dobi biokatalizatora lipaze postati biokatalizatori budućnosti.

BIODIESEL FUEL PRODUCTION THROUGH ENZIME TRANSESTERIFICATION

Abstract

Interest and idea of using renewable vegetable feeds as a replacement for mineral oils and the process of transesterification are over hundred years old. Today, for industrial production of biodiesel fuel, used are several processes of transesterification, achieving high conversions of triglicerides into corresponding methyl esters. Recently, there has been intense research of enzime catalyzed transesterification, which, due to its advantages, is becoming a growingly attractive manner of producing biodiesel fuel.

Introduction

Within global, consumerist economy, human needs are increasing more and more, and the requirements imposed upon chemical industrial processes are becoming more stringent as well. Limited character of the most significant energy resources and the adoption of the Kyoto Protocol (in 1999) have imposed commitments requiring new ways of applying environmentally tolerable technologies, safe production with regard to human health and safety, production processes with as low consumption of feeds and energy as possible, with a possibility of recycling, and the like.

Although today the share of renewable energy sources in total consumption in the EU amounts to around 6 %, a major increase is expected in the future, so that the 21st century is also called the century of renewable energy. (1)

Out of all renewable energy sources, the most possibilites are offered by the biomass, while biofuels produced out of biomass, apart from meeting EU Directives prescribing the introduction of such fuels, are taking up an increasingly significant position within the national economy of individual EU states. (2)

Today, various technologies are used in the industrial production of biodiesel fuel, most developed being the transesterification process with alkaline catalyst, achieving high conversions of fat/oil into corresponding esters over a short period. Since the cost effectiveness of these processes depends upon several important factors – e.g. feed quality i.e. composition, energy consumption, separation and purification of byproducts, wastewater processing, and the like, various improvement possibilities are being reviewed, including also biocatalytic conversion i.e. enzime technology.

Enzime transesterification

Enzime technology is an interdisciplinary area which OECD (Organization of Economic Cooperation and Development) has recognized as an important component of sustainable industrial development. Enzimes used in reactions with fatty acids, mono- and diacylglycerols and glycerol – lipases – are playing a key role in the degradation of lipides. (3, 4) Over the past around a dozen years, there has been an increased interest into the research of lipases, for the following three principal reasons:

1. enzime paradigm i.e. catalytic function of lipases: lipases, although water-soluble, catalyze reactions including insoluble lipide substrates on the interphase surface lipide-water, characterizing the unique structure of lipases,
2. importance of lipases for medicine, i.e. their significance in regulating metabolism,
3. discovery of lipases as tools for catalyzing not only hydrolysis, but also various reversible reactions, such as esterification, transesterification, and aminolysis in organic solvents.

Table 1: Industrial application of lipases (5)

Area	Application	Product
hydrolysis		
food	hydrolysis of milk (milk fats)	sweeteners for dairy products
Chemical production of fats	hydrolysis of oil and fat	fatty acids, diglycerides and monoglycerides, reagents for the analysis of lipides
Chemical production of surface active substances	analysis of fatty acids distribution in triglycerides, removal of oil stains, drops and lipides	laundry and household detergents
medicine	blood analysis of triglycerides	diagnostic tools
esterification		
Chemical production – specialty chemicals	synthesis of esters	chiral intermediaries, esters, emulsifyers
food-chemical and pharmaceutical production	transesterification of natural oils	oils and fats

Biocatalysts have some advantages with regard to classic catalysts: their specific character, regioselectivity and enantioselectivity, enabling catalytic reactions with less byproducts, lower waste treatment costs and reaction conditions which are milder in the sense of temperature and pressure.

Due to the property of preserving catalytic activity in organic solvents, tested were the activities of lipases as catalysts, in order to determine their potential for the conversion of excessive fat and oil into highly valuable products for nutrition and industrial use, while further examples were found in the resolution of racemic compounds, synthesis of pharmaceutical products and surfactants, bioconversion of oil and fat.

Biological function of lipases is the catalysis of ester hydrolysis, particularly long-chained triacylglycerols, as well as reverse reactions; esterification, transesterification, aminolyses, oximolyses and tiotransesterifications in anhydride solvents, biphase systems and micelar solutions. Examples of industrial application of lipases are listed in Table 1.

In order to make the use of lipases not only efficient, but also economically profitable in both water and non-water solvents, intensified was the research of their modification techniques, which may be classified into three categories: chemical modification, physical modification and genetic engineering, while they are performed for increasing the activity, selectivity or stability and improved solubility in organic solvents.

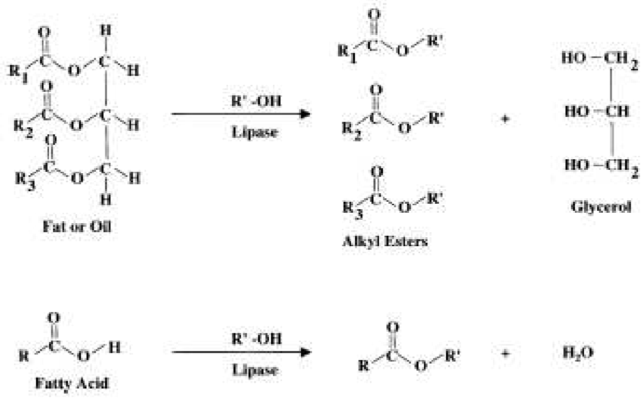
Physical modification most applied on lipases is immobilization - a process binding the enzime to the insoluble solid substrate, while the methods used may be classified in 3 groups: adsorption to the carrier material, incorporation and capsulation, and covalent binding to the active matrix.

Use of biocatalysts in the transformation of fat/oil, as well as glycerides and fatty acids, into highly valuable products has been documented by numerous papers, whose purpose was a more eficient use of immobilized lipases as cataysts for obtaining simple vegetable oil esters. (4, 5)

Production of biodiesel from unrefined feeds, such as used oil/fat, results in difficulties caused by the properties of such feeds and/or the composition of free fatty acids. That is why efforts are being made to improve the production process through investigating enzime alcoholysis of vegetable and animal oil/fat in both solvents and systems without solvents, using primary and secondary alcohols. The use of immobilized lipases for industrial purposes is most significant, especially now that the immobilized lipases may be reused, considerably cutting down catalyst costs.

When utilizing used edible oils from restaurants and fats as feeds for obtaining alkyl esters, the yields exceeded 95%, using different commercially available lipases. Used was the enzime IM ps-30, with a potential for producing biodiesel, with the yield of 98% of ester from the used restaurant fats, while earlier work using this enzime has shown that it may be used even several times. Figure 2 shows an example of esterification reactions with lipases as catalysts.

Figure 2: Esterification reaction with lipases



In esterification processes with lipases as catalysts, investigated were various types of alcohols - primary, secondary, straight-chained and branched, while the results of such processes i.e. the conversion achieved is shown in Table 2.

Short-chained alcohols (methanol, ethanol) change the properties of lipases, which may impose a negative impact on the reaction, which is why new ways of producing biodiesel are being tested, using methyl-acetate as acyl-acceptor, which – using the lipase Novozym 435 (Candida antarctica) – provides a 92% utilization level. (6,7)

Table 2: Conversion examples of some enzimes as catalysts in transesterification reactions (8)

Oil	Alcohol	Lipase	Conversion %	Solvent
Rapeseed	2-etil-1-hexanol	*Candida rugosa*	97	no
Mango	C_4-$C_{18:1}$-alcohols	*Mucor miehei* (Lipozyme IM-20)	86,8-99,2	no
Sunflower	ethanol	*Mucor miehei* (Lipozyme)	83	no
Fish	ethanol	*Candida antarctica*	100	no
Restaurant oils	ethanol	*Pseudomonas cepacia* (Lipase PS30) *Candida antarctica* (Lipase SP 435)	85.4	no
Tallow, soybean, rape seed	prim. alcohol.* sec. alcohol,** methanol, ethanol	*Mucor miehei* (Lipozyme IM60) *Candida antarctica (SP435)* *Mucor miehei* (Lipozyme IM60) *Mucor miehei* (Lipozyme IM60)	94,8-98,5 61,2-83,8 19,4 65,5	hexane hexane no no
Sunflower	methanol methanol ethanol	*Pseudomonas fluorescens*	3 79 82	no petrol eter no
Palmseeds	methanol ethanol	*Pseudomonascepacia* (Lipase PS 30)	15 72	no no

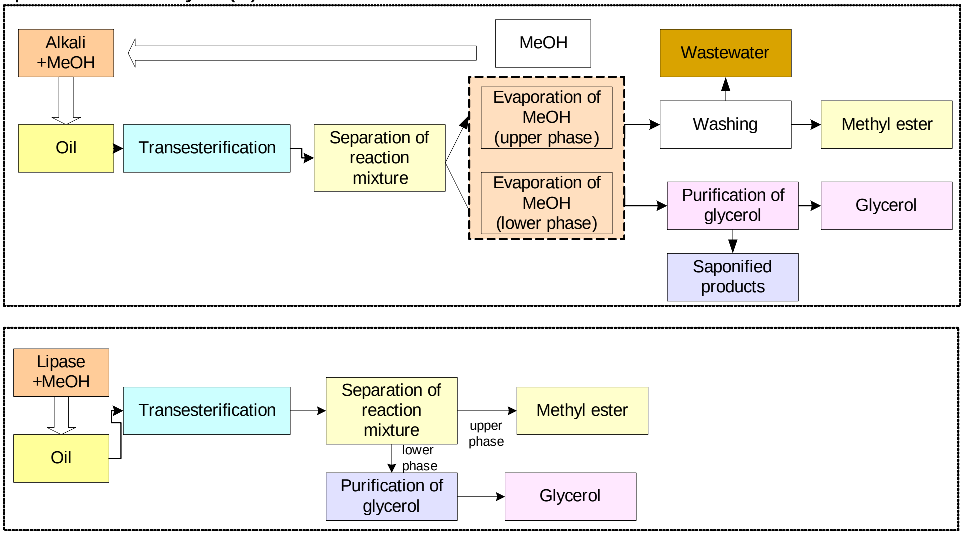
*methanol, ethanol, propanol, butanol, iso-butanol
**isopropanol, 2-butanol

The comparison between alkaline-catalyzed reaction and lipase-catalyzed reaction in biodiesel fuel production is given in Table 3.

Table 3: The compariosn between alkaline-catalyzed reaction and lipase-catalyzed reaction in biodiesel fuel production (8)

	Alkaline-catalyzed process	Process with lipses as catalyst
Reaction temperature	60º-70ºC	30º-40ºC
Free fatty acids in the feed (FFA)	soaps	methyl ester
Water in the feed	Interference with reaction	no impact
Methyl ester yield	high	high
Glycerol generation	difficult	easy
Methyl ester purification	multiple rinsing	no
Catalyst production	cheap	relatively expensive

Figure 3: Flow chart of biodiesel fuel production using alkaline catalyst and using lipases as catalyst (8)



Most used today in the commercial production of biodiesel fuel is the alkaline-catalyzed process, while the difficulties arising in it are predominantly associated with removing the catalyst from wastewater, evaporation of methanol, purification of glycerol, removal of saponified products, whereas, in the processes with lipases as catalysts they are not present, as shown in Figure 3: Compared production of biodiesel fuel using alkaline catalyst and using lipases as catalyst.

Due to the said advantages it is to be expected that further investigation of lipases as catalysts in the production of biodiesel fuel will continue to be most intense. The use, as well as the range of various biotechnological applications of lipases is growing, while a constant optimization of lipases and reaction conditions increasingly enables cost effective production of highly valuable chemicals and pharmaceutical products.

Conclusion

Biodiesel fuel is becoming increasingly attractive, due to its environmental properties and the fact that it constitutes a renewable energy source.

Specific character of reactions catalyzed by enzimes – lipases, results in the use of these enzimes in numerous processes of syntheses or modifications of oil and fat. Compared to conventional chemical processes, enzime catalysts successfully catalyze reactions at much milder conditions of temperature and pressure, while difficulties in the existing processes of producing biodiesel fuel, mostly associated with removing the catalyst from wastewater, evaporation of methanol, purification of glycerol, removal of saponified products ... are not present in the processes using lipases as catalysts.

Investigating biocatalysts in esterification reactions i.e. use of enzimes as catalysts, links – on the microlevel – the area of biotechnology with that of chemical engineering, capable of continuing the development and dimensioning of these processes to a larger scale, once the issue of price of this kind of catalysts is resolved, since it is for the time being still too high for industrial use.

Biotechnology and genetic engineering will most certainly play an important role in the production of lipases for industrial application, while the future will show whether, in the age of biocatalysts, lipases are to become the biocatalysts of the future.

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UDK	Ključne riječi:	Key words:
665.3.095.13	biodizelsko gorivo, metilni esteri masnih kiselina biljnih triglicerida	biodiesel fuel, vegetable oil fatty acids methyl esthers
66.098	enzimski katalitički postupci	enzimatic catalytic proceses
577.153	lipaza	lipase
665.3.094.942	transesterifikacija biljnih triglicerida	vegetable triglyceride transestherification
66.095.13	esterifikacija	esterification

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Primljeno / Received:
17.3.2006.