Enzymatic Synthesis of Fragrance Ester by Lipase from Marine Actinomycetes for Textile Industry

K. Selvam, B. Vishnupriya, M. Maanvizhi

Abstract ***The present study was carried out to investigate the enzymatic synthesis of fragrance ester from brewery industry effluent by lipase of S. acrimycini NGP 1, S. albogriseolus NGP 2 and S. variabilis NGP 3 which was isolated from the marine sediments of South Indian coastal region. The maximum conversion percentage of ester by lipase producing S. variabilis NGP 3 was 48.72 % and also a strong peak at 1745.21 cm-1 was observed by fourier transform infrared (FTIR) spectroscopy which indicated the presence of ester (C = O). The synthesized esters were imparted on the knitted fabric by exhaustion and microencapsulation method. In the qualitative evaluation of fragrance test for exhausted and microencapsulated knitted fabric, the judges were rated '2' (indicates poor) and '4' (indicates fair) respectively for the sensorial fragrance emitted from the fabric coated by the ester of S. variabilis NGP 3. In the quantitative evaluation, fragrance releasing percentage from exhausted and microencapsulated knitted fabric was found as 31.14 and 39.78 respectively on 48 hrs of treatment. Both qualitative and quantitative evaluation of fragrance test indicated that, the microencapsulated ester of S. variabilis NGP 3 on the knitted fabric emitted better fragrance than by exhausted fabric.***

Keywords- Ester, exhaustion, microencapsulation, knitted fabric.

I. INTRODUCTION

The marine actinomycetes are substantiated by culture independent studies. The majority of the microorganisms from the natural environment resist cultivation in the laboratory. Recent studies have shown that, the majority of actinomycetes from the marine environments such as sediments and sponges are recovered by culture independent method not by cultivation - based methods which have established that the novel actinomycetes will facilitate the investigation of the ecological roles and provide an important source for discovery of novel metabolites [1] [2]. Actinomycetes have been detected in an unique marine environment such as in marine organic aggregates and deep - sea gas hydrates reservoirs, where they were found to be the major components of the microbial communities [3]. The findings confirm the presence of indigenous marine actinomycetes in the oceans and indicate that marine actinomycetes are widely distributed in different marine environments and habitats. Both culture dependent and independent methods demonstrate that novel actinomycetes found everywhere in the oceans from deep floor to coral reefs.

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The extensive variation among the different marine environments, the presence of actinomycetes that are distantly related to known taxa in larger amount [4]. Many commercially important extra and intra cellular enzymes such as amylases, protease, cellulase, lipase, keratinase, xylanase and L-asparaginase were produced from marine actinomycetes [5] [6]. Among them lipase gain more attention in industrial applications especially in the ester production.

Lipases (triaycl glycerol acyl hydrolases, EC 3.1.1.3) catalyze the hydrolysis and the synthesis of esters from glycerol and long chain fattyacids [7]. They are obtained from microorganisms which produce a wide variety of extracellular lipases [8]. Lipases are active in organic solvents where they catalyst a number of useful reactions including esterification, transesterification, regioselective acylation of glycerols, synthesis of peptides and other chemicals [9]. They are mostly inducible enzyme and require some form of oil, fattyacid, fattyacid alcohol or fattyacid ester and surfactant for induction [10]. Recently, the interest in microbial lipase production has increased [11], because of its large potential in industrial applications as additives for foods (flavor modification), fine chemicals (synthesis of esters), waste water treatment (decomposition and removal of oil substances), cosmetics (removal of lipids), pharmaceuticals (digestion of oil and fats in foods), leather (removal of lipids from animal skins) and medical (blood glyceride assay) fields [12] [13] [14]. Other useful features such as broad substrate specificity, the versatility of the molecular structure and stability in organic solvents [15]. Alcoholic esters of short chain fatty acids are important aroma compounds, whereas esters of long chain fatty acids are being explored for their use as fuel (biodiesel) and as waxes [16]. In the production of flavor esters, reaction occurred between short chain acid and alcohol. The process involved is an esterification reaction whereby alcohol reacted with carboxylic acid with the elimination of water. Ethyl, isobutyl, amyl and isoamyl acetates frequently used as components in flavouring and isopropyl, benzyl, octyl, geranyl, linalanyl and methyl acetates were important additives in perfumes [17]. Flavor esters were generally produced by free and immobilized lipases from various sources in organic solvents [18]. Until now, there are several research have been carried out in synthesizing flavor ester such as ethyl valerate, hexyl acetate, isoamyl alcohol and benzyl acetate [19]. Biosynthesis of esters can be synthesized by the following ways such as enzyme, microorganisms, plant cell and cultures of tissues. Among them, enzyme based synthesis of ester is more efficient and most frequently used technique. Hence, the present study aims to synthesis of fragrant esters by lipase of marine actinomycetes and applied on fabrics by exhaustion and microencapsulation method.

II. MATERIALS AND METHODS

A. Collection of the effluent

Brewery industry effluent 1000 ml was collected from Siva distilleries, Coimbatore, Tamilnadu, India. The effluent (50 ml) was taken in 250 ml of Erlenmeyar flask and added with 50 ml of butyric acid (50 mM) and lipase (5 %). The reaction mixture was incubated at 37ºC for 24 hrs in a horizontal water bath shaker at 150 rpm. 1.0 ml sample was withdrawn and terminated the reaction with 10 ml of ethanol: acetone (1:1 v/v). To this 2 - 3 drops of phenolphthalein indicator was added and titrated against 0.01 M NaOH. Endpoint is the appearance of pink colour. The yield of ester was expressed in per cent [20] [21]. Control was run simultaneously without adding enzyme in the reaction mixture.

Calculation

$$\text{Conversion percentage} = \frac{\text{Vol}_{NaOH}(\text{without enzyme}) - \text{Vol}_{NaOH}(\text{with enzyme})}{\text{Vol}_{NaOH}(\text{without enzyme})}$$

B. Identification of reaction product

Identification of ester was analyzed by TLC. The sample was spotted on the silica gel plate and the solvents hexane and diethyl ether was used as a mobile phase at the ratio of 9:1 (v/v). The developed spots were identified by spraying the rhodomine B (0.5 % w/v in ethanol) on the plate and it was further analyzed by fourier transform infrared (FTIR) spectroscopy. The spectral data obtained were compared with the reference chart to identify the functional groups present in the sample.

C. Process parameters Effect of time

The effluent (10 ml) was taken in 100 ml of conical flask and added with 10 ml of butyric acid (50 mM) and lipase (5 %). Incubate the reaction mixture at 37ºC in a horizontal water bath shaker at 150 rpm for 7 hrs. The reaction was assayed at the regular time interval of every one hour (1 to 7 hrs). Ester conversion percentage was determined.

Effect of temperature

The reaction mixture was incubated at various temperature ranges (30, 37, 45, 50 and 55ºC) at the shaking speed of 150 rpm and calculated the ester conversion percentage.

Effect of enzyme concentration

The reaction mixture was catalyzed at the different concentration of enzyme (5, 10, 15, 20 and 25 % v/v) and the conversion was determined.

D. Application of fragrant ester on fabric Sample preparation

Six pieces of steriled knitted fabric (30 X 20 cm) were selected for this study. Ester was coated on the fabric by exhaustion and microencapsulation method.

Exhaustion and microencapsulation method

Ester was coated on the three pieces of fabric using 8.0 per cent citric acid as binder at 50ºC and incubate it for 30 min. The fabric was tested for its fragrance property [22]. Microcapsules were prepared by taking equal volume of sodium alginate (3 %) and ester solution in a 250 ml Erlenmeyer flask. The total content was sprayed into calcium chloride solution by sprayer. The droplets were retained in calcium chloride for 15 min and washed with iso propyl alcohol followed by drying at 45°C for 12 hrs. After that, the microcapsules were coated on the remaining fabric [23].

Qualitative evaluation

Three male panelists were selected for this study. The exhausted and microencapsulated fabrics were to be worn on a specific foot of the each panelist [24]. At the end of a day, panelists reported to the lab to remove the fabric, seal them in plastic bags, so that there was no release of fragrance due to air or light. This study was carried out with the same individuals for 48 hrs. Odor evaluation was made by four judges, in the individual grading sheet ranges from 0 to 10 ("repulsive to ideal odor").

Quantitative evaluation

Releasing rate of fragrance was measured using UV/Visible spectrophotometer [24]. Fragrance was extracted from 1.0 g of sample (knitted fabric) with ethanol for 3 min at 40°C and ensured complete evaporation of ethanol. Extracted fragrance solution was diluted to 1:10 ratio with distilled water and measured the absorbance at 260 nm. Ethanol was used as a blank.

III. RESULTS AND DISCUSSION

A. Synthesis of ester

Lipase from marine actinomycetes catalyzed the esterification of fatty acids. The conversion per cent of ester was calculated by comparing the reacted fatty acid with the total fatty acid in the reaction mixture. The conversion per cent of ester by lipase producing actinomycetes were found to be 20.03, 27.78 and 48.72 per cent. The pure synthesized ester was detected as violet spot on the silica gel plate after the dye rhodomine B was sprayed on TLC plate. The synthesized ester from *S. acrimycini* NGP 1 and *S. albogriseolus* NGP 2 were further analyzed by fourier trasform infrared spectroscopy (FTIR). According to the table 1, 2 and figure 1, 2 small peak was observed at 1742.19 cm^{-1} and 1689.02 cm^{-1} which indicated the functional group of esters (C = O stretch). Ester from *S. variabilis* NGP 3 was observed in FTIR (Table 3; figure 3), a strong peak at 1745.21 cm^{-1} which indicated the presence of ester (C = O). Followed the strong peak, two week peaks were also observed to be 1076.28 and 1226.73 cm^{-1}, which indicated the presence of ester group (C - O).

In an earlier report, the synthesis of methyl butyrate and octyl acetate through immobilized *Rhizopus oryzae* NRRL 3562 lipase mediated transesterification was studied under solvent free conditions. The effect of different transesterification variables, namely, molarity of alcohol, reaction time, temperature, agitation, addition of water and enzyme amount on molar conversion (%) was investigated [25].

B. Effect of time

The effect of time was the important parameter for obtaining high production yield of ester. The production of ester at various time intervals is presented in figure 4. The results showed that, the ester conversion percentage was increased gradually from 1 to 6 hrs. Ester conversion percentage of *S. acrimycini* NGP 1 was maximum on 6 hrs of time interval and yield was 48.0 per cent. In addition, *S. albogriseolus* NGP 2 and *S. variabilis* NGP 3 ester conversion percentage

was found maximum (55.0 and 70.0 %) on 5 hrs of time interval. After 5 hrs of reaction time, the yield of ester was started to decrease. The results showed similarly to the study of Gulati *et al*., 2003, the high conversion percentage of ester was obtained in only 12 hrs of time interval. The optimum conditions to produce butyl acetate, a pine apple flavor was at reaction time 18 hrs, temperature at 37°C and amount of enzyme 25.0 per cent via lipase-catalyzed reaction with the substrate butanol and acetic acid [20].

C. Effect of temperature

Effect of varying reaction temperature (30 to 55°C) for the enzymatic synthesis of ester is shown in figure 4. Initially, the percentage conversion of ester was increased with increasing temperature from 30 to 50°C. The highest percentage of ester was obtained on 50°C by all the three marine actinomycetes. The yield was found to be 42.05, 50.10 and 68.33 per cent by the respective actinomycetes. Above 50°C the conversion was decreased drastically. Lipase from *Rhizopus arrhizus* showed the esterification activity at the optimum temperature of 30°C; surfactant-coated lipase catalyzed for the esterification reaction with the substrate palmitic acid and glycerol gave maximum conversion of 74.02 per cent. Immobilized *A. terreus* lipase-catalyzed esterification of sorbitol with stearic acid in n-hexane on optimizing various physico-chemical conditions [21].

D. Effect of enzyme concentration

Effect of varying the amount of enzyme in the reaction was influenced the ester conversion percentage. The results were showed in figure 4. The results revealed that, the different concentration of lipase enzyme in the range of 5 to 25 per cent were added separately in the reaction mixture. The ester conversion percentage was found maximum at 5 to 15 per cent of enzyme concentration; the yield of ester from *S. acrimycini* NGP 1, *S. albogriseolus* NGP 2 and *S. variabilis* NGP 3 was found to be 40.05, 48.79 and 63.33 per cent. In the previous study, reaction mixture for direct esterification and transesterification was carried out at 45ºC in equimolar concentration of substrates: acid or ester and alcohol [26]. When the concentration of the enzyme increased, the yield was decreased. A simple kinetic model derived from a Ping - Pong mechanism is proposed to describe the mono-esterification of glucose with stearic acid catalyzed by immobilized lipases from *Candida sp.* [27].

E. Application of fragrant ester on textile industry

The fragrant ester synthesized by the lipase mediated enzymatic reaction was imparted on the knitted fabric by exhaustion and microencapsulation method. In an exhaustion method, the ester was finished on piece of knitted fabric using 8 per cent citric acid as a binder. Besides, the synthesized ester was microencapsulated with calcium chloride and finished on the selected knitted fabric. Both treated fabrics were subjected for qualitative evaluation of fragrance finishing. In the previous study, ester from *Bacillus* was coated on the fabric using 8.0 per cent citric acid as binder at 50ºC and incubates it for 30 min and tested its fragrance property [22]. Besides, ester from lipase producing *B. cereus* was coated on the steriled cotton fabric by exhaustion method using sodium alginate as an encapsulator [23].

Qualitative evaluation

Qualitative evaluation test was conducted for the exhausted and microencapsulated fabrics to assess the performance of fragrance finishing on the tested fabrics. Qualitative evaluation of odor control for exhausted and microencapsulated fabrics were presented in table 4 and 5. Odor evaluations were made by four judges, after the removal of fabrics from the foot of three male panelists on 48 hrs. The rating of the test was ‘10’, ‘9’, ‘8’, ‘7’, ‘6’, ‘5’, ‘4’, ‘3’, ‘2’, ‘1’ and ‘0’. The rating ‘10’ indicates ideal and ‘0’ shows repulsive odor.

Interpretation

The qualitative evaluation of odor control for exhausted knitted fabric (Table 4) showed that, the four judges were rated ‘2’ (indicates poor) as the maximum average value of the fragrance emitted from the fabric in which imparted ester was synthesized from *S. variabilis* NGP 3 than esters from *S. acrimycini* NGP 1 and *S. albogriseolus* NGP 2. It is observed in microencapsulated knitted fabric (Table 5), the judges rated ‘4’ (indicates fair) for the fragrance emitted from the fabric which is coated by esters synthesized from *S. variabilis* NGP 3. The ester from *S. variabilis* NGP 3 was emitted fair fragrance than the esters from the respective actinomycetes. The qualitative evaluation indicated that, the microencapsulated ester on the knitted fabric emitted better fragrance than by exhausted fabric.

Quantitative evaluation

In the quantitative evaluation, fragrance releasing percentage of the exhausted knitted fabric was observed immediately was 7.70, 10.15 and 22.20 respectively by the esters synthesized from *S. acrimycini* NGP 1, *S. albogriseolus* NGP 2 and *S. variabilis* NGP 3. An immediate observation of the fragrance from knitted fabric which was microencapsulated by the esters from the respective actinomycetes were 11.24, 15.19 and 27.87 per cent. The quantitative evaluation of odor control was carried out, after the removal of fabrics from the foot of three male panelists on 48 hrs. In this observation, fragrance releasing percentage of exhausted knitted fabric was increased than immediate observation. It was found to be 11.14, 15.09 and 31.14; whereas in microencapsulated knitted fabric, fragrance releasing percentage was found as 17.18, 21.07 and 39.78. The values were increased a fold than an immediate observation (Table 6). The results showed similarly to the study that, releasing rate of fragrance was measured using UV/Visible spectrophotometer. Fragrance was extracted from fabric with ethanol for 3 min at 40°C. Extracted fragrance solution was measured the absorbance at 260 nm [24].

IV. CONCLUSION

In the present study, the fragrant ester synthesized by the *S. variabilis* NGP 3 lipase mediated enzymatic reaction was exhibited maximum production of ester than others. The synthesized esters were imparted on the knitted fabric by exhaustion and microencapsulation method. Based on the methods, microencapsulated ester emitted better fragrance which was proved by qualitative and quantitative evaluation of fragrance test.

Table 1: FTIR spectra analysis of ester from S. acrimycini NGP 1

Absorption ranges (cm^{-1})	Types of vibration	Functional group names
1369.46	N = O stretch	Nitro group
1652.46	C-C=C stretch	Alkene
1742.19	C = O stretch	Esters
3206.35	N - H stretch	Amide
3819.20	N-H stretch	Amide
3522.23	O - H stretch	Phenols and alcohols

Table 2: FTIR spectra analysis of ester from S. albogriseolus NGP 2

Absorption ranges (cm^{-1})	Types of vibration	Functional group names
1253.33	C - O stretch	Ethers
1445.12	H-C-H stretch	Alkanes
1473.39	C – C = C stretch	Aromatic ring
1689.02	C = O stretch	Esters
2705.25	C - H stretch	Aldehyde
2717.18	C - H stretch	Aldehyde
3389.90	O - H stretch	Carboxylic acid

Table 3: FTIR spectra analysis of ester from S. variabilis NGP 3

Absorption ranges (cm^{-1})	Types of vibration	Functional group names
1076.28	C - O stretch	Esters
1226.73	C - O stretch	Esters
1369.46	N = O stretch	Nitro group
1512.19	N - H Bend	Amine secondary
1745.21	C = O stretch	Esters
2927.38	O - H stretch	Carboxylic Acid
3371.57	N - H stretch	Amide
3838.34	O - H stretch	Phenols and alcohols

Table 4: Qualitative evaluation of fragrance ester in exhausted knitted fabric

Subjects	Height (cm)/ Weight (Kg)	Sa Judge I	II	III	IV	Average	Sag Judge I	II	III	IV	Average	Sv Judge I	II	III	IV	Average
Subject 1 (Male / 39 yrs)	174/58	0	0	1	0	0	1	1	1	0	1	2	1	2	2	2
Subject 2 (Male / 39 yrs)	178/62	1	0	1	1	1	0	1	1	0	1	2	2	3	2	2
Subject 3 (Male / 39 yrs)	159/54	0	0	1	1	1	1	0	1	1	1	2	3	2	2	2

Table 5: Qualitative evaluation of fragrance ester in microencapsulated knitted fabric

Subjects	Height (cm)/ Weight (Kg)	Sa Judge I	II	III	IV	Average	Sag Judge I	II	III	IV	Average	Sv Judge I	II	III	IV	Average
Subject 1 (Male / 39 yrs)	174/58	1	0	1	1	1	1	2	1	0	1	4	4	4	5	4
Subject 2 (Male / 39 yrs)	178/62	0	1	1	0	1	1	2	2	0	2	4	5	5	4	4
Subject 3 (Male / 39 yrs)	159/54	1	1	1	0	1	0	2	1	1	1	4	5	4	4	4

Yrs: Years; Sa: *Streptomyces albogriseolus* NGP 1 ; Sag:*Streptomyces albogriseolus* NGP 2*;* Sv: *Streptomyces variabilis* NGP 3

Interpretation:

0 -Repulsive ; 1 - Very Poor ; 2- Poor ; 3 - Poorly Fair 4 - Fair ; 5 - Acceptable; 6 - Fairly Good; 7 -Good ; 8 - Very Good ; 9 - Excellent ; 10- Ideal

Table 6: Quantitative evaluation of fragrance ester in knitted fabric

Fabric	Releasing percentage of fragrance (%)		
	S. acrimycini NGP 1	*S. albogriseolus* NGP 2	*S. variabilis* NGP 3
Immediate observation			
EKF	7.70	10.15	22.20
MEKF	11.24	15.19	27.87
After 48 hours			
EKF	11.14	15.09	31.14
MEKF	17.18	21.07	39.78

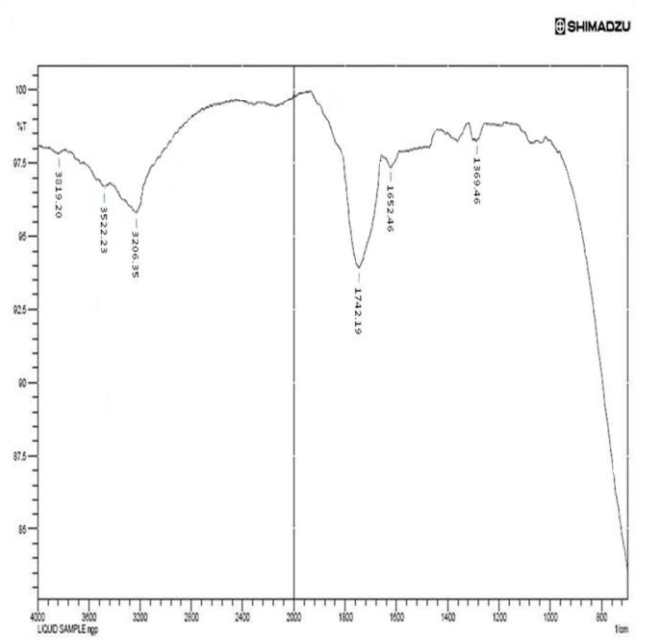

Fig1: FTIR analysis of ester compound by lipase producing S. acrimycini NGP 1

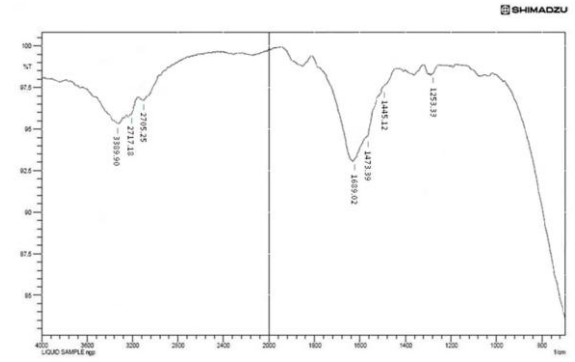

Fig. 2:FTIR analysis of ester compound by lipase producing S. albogriseolus NGP 2

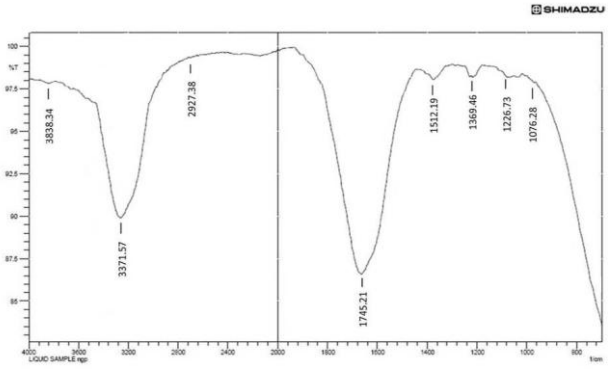

Fig. 3: FTIR analysis of ester compound by lipase producing S. variabilis NGP 3

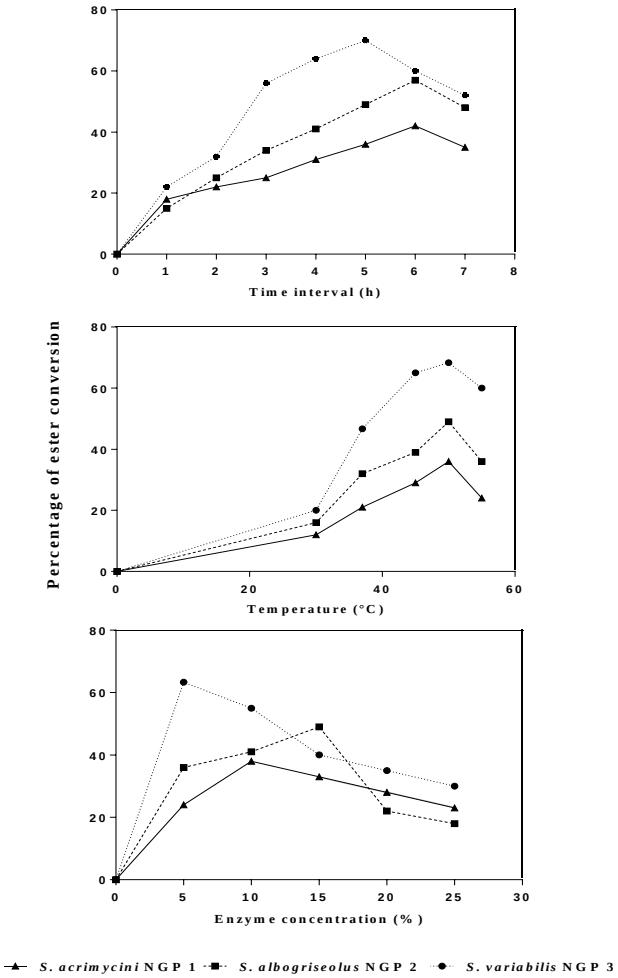

Fig. 4: Effect of time, temperature and enzyme concentration on ester conversion

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