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Thermal and Reused[1] Stability of Immobilized Lipase in Carrageenan

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143 Thermal and Reused[1] Stability of Immobilized Lipase in Carrageenan

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144 Abstract. Lipase is one kind of enzyme that is widely used[4] as a catalyst in industrial and medical. Lipase has a great catalytic ability, but is easily affected by temperature, and is also difficult to separate at the end of the reaction. This[5] causes most of lipase[6] used only in one reaction cycle. Therefore an enzyme immobilization is needed,[7] so that lipase can[8] maintenance it's

activity in high temperatures, and can also be separated at the end of the reaction so that it can be re-used. This study aimed to determine the thermal stability and re-used stability of the immobilized lipase in carrageenan. The method used for immobilizing lipase is through entrapment method. The effectiveness of immobilized lipase tested through the hydrolysis reaction of palm oil. The result was found that the immobilized lipase in carrageenan was able to maintain its catalytic activity up to 50 oC and also up to five reaction cycles.

Keywords. Lipase, carrageenan, immobilization, thermal stability, reused stability

Introduction

Enzymes are functional protein units that play a role in catalyzing reactions in cell metabolism and other reactions in the body. Spesifications of enzyme in substrate are very high in accelerating chemical reactions.

Lipase is one kind of enzyme that have potential properties to be utilized, including large catalytic power and specificity to the substrate of the reaction catalyzed[1]. Lipase carry out their catalytic activities by reducing activation energy and accelerating the achievement of fidelity but not changing the equilibrium point of the reaction[2].

Although it has a very large catalytic ability, activity of lipase is highly dependent on environmental conditions. It takes an optimum temperature condition so that the enzyme can work optimally. Conditions like too high temperatures can cause the enzyme to become damaged so that its catalytic activity decreases. Besides being easily affected by changes in temperature,

enzymes also difficult to separate at the end of the reaction making it difficult to reuse[3].

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To overcome the weakness of the enzyme, a method is needed to modify the enzyme, so that the enzyme is not easily damaged due to temperature and is also easy to separate at the end of the reaction. Enzyme immobilization is one kind of the enzyme modifications by binding or attaching enzymes physically and chemically to the supporting solids (immobilizing matrix) like chitosan, carrageenan, zeolite, etc which are insoluble in water. By attaching enzymes to the solid support, it is expected that the enzyme will become stronger, resistant to changes in reaction conditions so that it can be used repeatedly and can improve the reaction results[3].

Carrageenan is a group of galactose polysaccharides extracted from seaweed. Carrageenan has the ability to form a gel thermoreversible which is melted if heated and forms a gel again if cooled[4]. Based on the carrageenan ability, it is expected that carrageenan is able to bind or hold lipase enzymes in the cavity formed when carrageenan is heated, so it is interesting to learn about immobilization of lipase enzymes through entrapment method using carrageenan. The effectiveness of immobilization was observed by the hydrolysis reaction of palm oil.

Materials and Method

Materials and equipment

The materials used in this study were palm oil, carrageenan, pancreatic lipase enzyme, Whatman 41 filter paper, pH indicator paper, aquadest, n-hexane, ethanol, sodium hydroxide (NaOH), sodium monohydrogen phosphate (Na2HPO4.2H2O), sodium dihydrogen phosphate (NaH2PO4.H2O), biuret reagent, indicator phenolphthalein, serum albumin bovine (BSA). The equipment used in this research is a set of glassware (Pyrex), shakers, ELISA reader, 50 mL Falcon bottles, pH meters, magnetic stir bar, glass funnel, burette, micropipette, analytical balance, oven, refrigerator.

Standardization of NaOH

Standardization is carried out by means of 0.05 grams of oxalic acid inserted in Erlenmeyer then added 25 mL of distilled water. The next solution is added 2-3 drops of phenolphthalein indicator and titrated with NaOH. Normality of NaOH is determined by the formula:

: weight of oxalic acid (gram)

: Volume of NaOH (mL)

0,126 : Molecular weight of oxalic acid (g/mmol) N : Normality of NaOH

(1)

Measurement of protein concentration

The standard solution is made by dissolving 100 mg of Bovin Serum Albumin (BSA) in a 10.0 mL measuring flask with the addition of distilled water to the boundary mark, so that the BSA standard solution is 10 mg / mL. From a standard solution of 10 mg / mL BSA, multilevel dilution was carried out with distilled water so that standard solutions were obtained with concentrations of 8, 6, 4, 2 mg/mL. Furthermore, each concentration of standard solution was taken 100 µL and added 160 µL of reagent biuret, mixing in ELISA plate. The mixture was then allowed to stand for 10 minutes then read the absorbance at λ 630 nm with a mixture of 100 µL aquadest and 160 µL biuret. The results of absorbance of standard solutions are used to make a standard curve (absorbance vs. concentration). Measurement of protein concentration was carried out using 100 µL of a protein solution plus 160 µL of a biuret reagent then carried out in the same manner as making a standard curve. The sample protein concentration is obtained by entering the sample absorbance data into the standard curve equation[5]:.

Lipase activity assay

As much as 1 gram of palm oil is put into a 10 mL volumetric flask and 10 µL of aquadest are added and then added n-hexane to the boundary mark. The solution was then transferred into a 50 mL falcon

bottle and added 150 mg of free lipase enzyme. The solution is stirred in a shaker incubator for 5 hours at 37oC. To control the reaction, palm oil is used without the addition of lipase enzymes with the same procedure. The reaction results are filtered then the filtrate plus 10 mL ethanol and 2-3 drops of the phenolphthalein indicator. Lipase enzyme activity was measured by determining the free fatty acids formed using a standardized 0.05 M NaOH solution. Calculation of activity units and specific activities uses the following formula[6]:

(2)

(3)

(4)

MW : molecular weight (g/mmol) W : weight (gram)
V : Volume of NaOH (mL)
N : Normality of NaOH

Lipase immobilization

The general immobilization procedure is by means of 500 mg carrageenan dissolved with 15 mL physiological NaCl then heated to a temperature of 70oC then cooled to 37 oC. Then 5 mL of 1% lipase enzyme solution was heated to 37 oC. After the carrageenan solution and enzyme solution reach the same temperature, the two solutions are mixed and stirred until smooth and then cooled. From this result, the enzyme is immobilized in carrageenan and the rest of the enzyme solution [6]. The resulting filtrate measured the protein content in accordance with the procedure for measuring protein concentration. The amount of immobilized lipase enzyme is the result of reducing the amount of the initial enzyme minus the amount of residual enzyme [7].

(5)

Activity assay of immobilized lipase

Immobilized lipase enzyme activity test was carried out through hydrolysis reaction. The method used is the same as the free lipase enzyme activity test method. However, free lipase enzymes are replaced with immobilized lipase enzymes [6].

Stability assay of immobilized lipase

The thermal stability test was carried out by heating the free lipase enzyme and the lipase enzyme immobilized at 35, 40, 45, and 50 oC for 20 minutes. After the enzyme is heated, it is then used in the oil hydrolysis reaction. In the repeated use stability test, lipase enzymes and free lipase enzymes that have been used in hydrolysis reactions are separated from the substrate, then reused for subsequent hydrolysis reactions [6].

Result and Discussion

Protein standard curve

Protein Standard Curve

0,6

0,5

0,4

0,3

0,2

y = 0,0484x + 0,0371

0,1 R² = 0,9903

0

0 2 4 6 8

10

12

Protein Concentration (mg/mL)

Absorbance

Protein standard curve has been made by using Bovin Serum Albumin with various concentration[96], then added with a biuret reagent and[97] measured the absorbance using ELISA reader at a wavelength of 630 nm. From the results of the concentration vs absorbance plot, the line equation y = 0.0484x + 0.0371 with R2 = 0.9903. This equation is then used[98] to calculate the levels of the initial protein and the remaining protein after enzyme immobilization.

Figure 1. Protein standard curve

Thermal stability of immobilized lipase

Lipase has optimum stability at certain[99] temperatures. The thermal stability assay of lipase aims to determine how the temperature[100] affects lipase activity in hydrolyzing palm oil. The reaction rate will increase as the temperature rises to its optimum limit because the enzyme will deactivate at a high[101] temperature [8]. The initial stage of the thermal stability test of lipase was carried out by heating lipase and immobilized lipase with temperature variations and then observed the effect on the hydrolysis activity. The actual thermal stability assay can also be done through reaction temperature variations because the increasing temperature can affect the hydrolysis reaction of palm oil. Temperature variations were carried[102] out at normal[103] temperatures (at 27 oC,

without preheating), 35 oC, 40 oC, 45 oC, and 50 oC. The results of thermal stability assay of lipase are presented in Figure 2 and 3.

Free Fatty Acid produced by Lipase

25

20

15

10

5

0

27

35

40

Temperature (0C)

45

50

Free Lipase Immobilized Lipase

Thermal Stability

0,045

0,04

0,035

0,03

0,025

0,02

0,015

0,01

0,005

0

0,0373

0,039

0,0332

0,0209

0,0175

0,0183

0,0187

0,0099

0,0025

25 30 35

40

Temperature (oC)[107]

45

50

55

Free Lipase Immobilized Lipase

0,0140

Specific Activity (U/mg)

Free Fatty Acid (%)

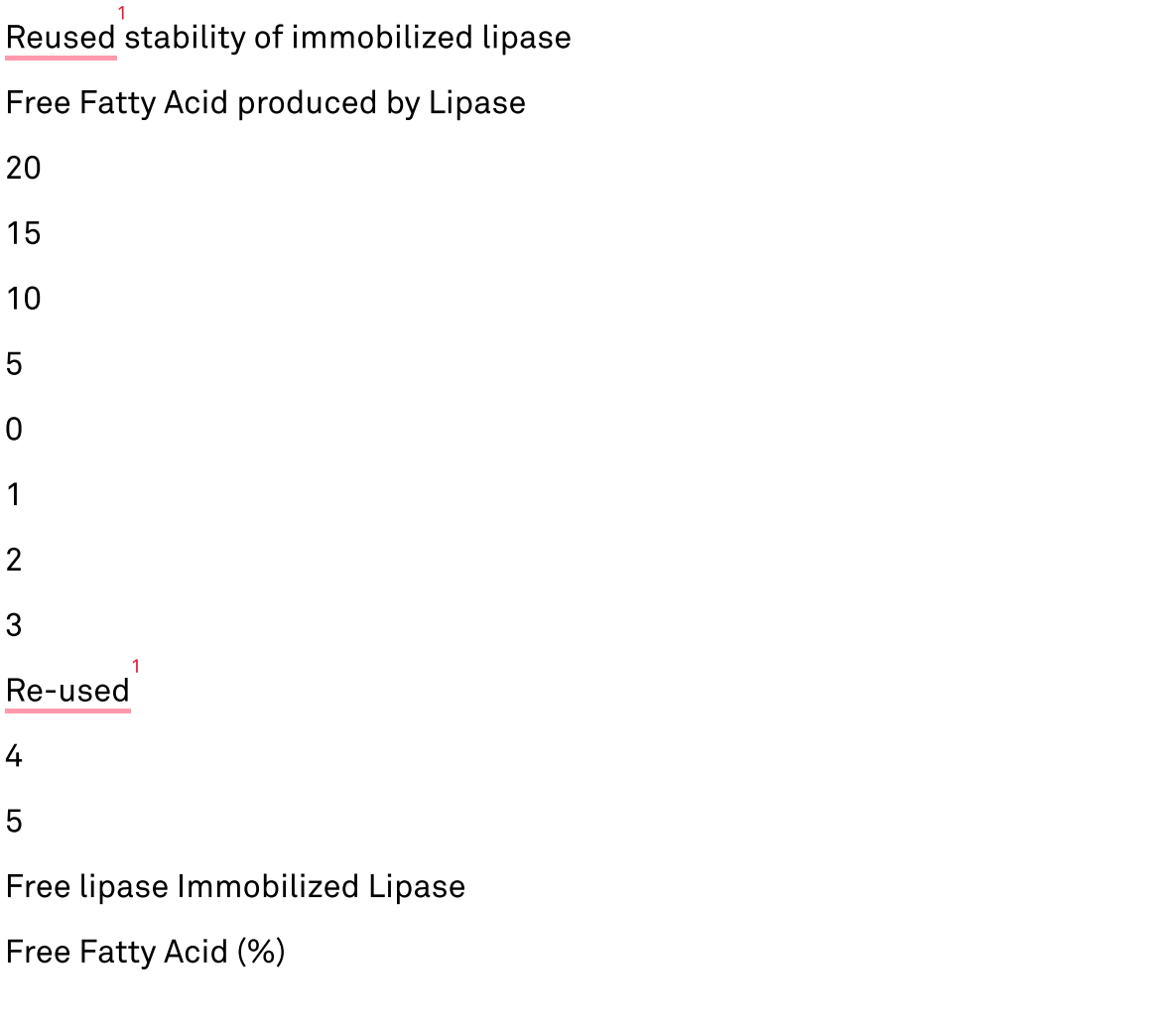
Figure 2. Free fatty acid (FFA) produced by lipase (thermal stability)

Figure 3. Thermal stability

Thermal stability of can represents the stability of immobilized enzymes. Enzymes with better thermal stability allow enzymes to be used in high temperatures[6]. From the picture above can be seen that lipase has the highest specific activity at 40 oC This is because the temperature is closest to the optimum temperature of the enzyme (37 oC). Heating treatment at 40 degrees showed the highest activity of free lipase and immobilized lipase. This shows that at this temperature the lipase structure has better interaction with the substrate[6]. At temperatures below the optimum temperatures, enzyme conformation is still not ready to catalyze the hydrolysis reaction of palm oil, so the specific activity becomes lower. At temperatures above the optimum temperature, the enzyme will get denaturation because the confirmation is damaged at high temperatures, so the activity is specific also decrease[10]. Immobilized lipase in carrageenan can maintenance its specific activity at high temperatures. This is consistent with the research conducted by Wardoyo[10].

Temperature treatment can cause the opening of protein structures and loss of enzyme activity[9]. The enzyme immobilized in supporting solids will increase its thermal stability because the supporting solids will protect the enzyme from

denaturation due to heat [11]. Immobilization causes lipase to be trapped in carrageenan which protects enzymes from temperature denaturation, so that enzyme conformation is maintained [9]. Therefore the enzyme is able to maintain its activity at high temperatures.



One purpose of enzyme immobilization is to find out whether the immobilized enzyme can be reused after being used in a reaction. The higher the rate of repeated use means the better the immobilization method used [5]. The results of reuse stability asssay of lipase are presented in Figure 4 and 5.

Reused Stability

0,04

0,035

0,03

0,025

0,02

0,015

0,01

0,005

0

0,0347

0,0191

0,0166

0,0160

0,0056

0,0078

0,0021

0 1 2 3 4 5 6

Reuse

Free Lipase Immobilized Lipase

0,0031

0,0114

0,0135

Specific Activity (U/mg)

Figure 4. Free fatty acid (FFA) produced by lipase (reused stability)

Figure 5. Reused stability

Based on figure 3, can be seen that immobilized lipase on carrageenan has better-reused stability if it compared with free lipase. Immobilized lipase on carrageenan is able to maintain its activity up to five times the reaction cycle. The results obtained are as expected, that immobilized lipase enzymes can be reused even with decreased activity.

Conclusion

Immobilized lipase in carrageenan has better stability than free lipase. Immobilized lipase in carrageenan improves the thermal stability up to 40 0C. Immobilized lipase in carrageenan also can maintain its activity up to five times reaction cycles, which is much better than free lipase.

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008/UNIMUS.J/PJ/PG
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1.	*Reused; re-used; reused; reuse; Re-used*	Text Inconsistencies	Correctness
2.	*was downloaded*	Passive Voice Misuse	Clarity
3.	~~doi~~ → DOI	Misspelled Words	Correctness
4.	*is widely used*	Passive Voice Misuse	Clarity
5.	*This*	Intricate Text	Clarity
6.	the lipase	Determiner Use (a/an/the/this, etc.)	Correctness
7.	needed,	Punctuation in Compound/Complex Sentences	Correctness
8.	can provide	Incomplete Sentences	Correctness
9.	an activity	Determiner Use (a/an/the/this, etc.)	Correctness
10.	temperatures,	Punctuation in Compound/Complex Sentences	Correctness
11.	*be re-used*	Passive Voice Misuse	Clarity
12.	the entrapment	Determiner Use (a/an/the/this, etc.)	Correctness
13.	*was found*	Passive Voice Misuse	Clarity
14.	stability.	Closing Punctuation	Correctness
15.	~~Specifications~~ → Specifications	Misspelled Words	Correctness
16.	the enzyme, or an enzyme	Determiner Use (a/an/the/this, etc.)	Correctness
17.	the substrate, or a substrate	Determiner Use (a/an/the/this, etc.)	Correctness

18.	~~large~~ → considerable, massive, immense	Word Choice	Engagement
19.	~~carry~~ → carries	Faulty Subject-Verb Agreement	Correctness
20.	~~a very large~~ → a tremendous, a considerable, a huge, an immense	Word Choice	Engagement
21.	the activity	Determiner Use (a/an/the/this, etc.)	Correctness
22.	~~difficult~~ → challenging	Word Choice	Engagement
23.	*be used*	Passive Voice Misuse	Clarity
24.	~~licence~~ → license	Mixed Dialects of English	Correctness
25.	~~work~~ → book	Word Choice	Engagement
26.	, and	Comma Misuse within Clauses	Correctness
27.	~~licence~~ → license	Mixed Dialects of English	Correctness
28.	~~doi~~ → DOI	Misspelled Words	Correctness
29.	*To overcome the weakness of the enzyme, a method is needed to modify the enzyme, so that the enzyme is not easily damaged due to temperature and is also easy to separate at the end of the reaction.*	Wordy Sentences	Clarity
30.	*To overcome the weakness of the enzyme*	Misplaced Words or Phrases	Correctness
31.	enzyme,	Punctuation in Compound/Complex Sentences	Correctness
32.	etc.	Comma Misuse within Clauses	Correctness
33.	*Enzyme immobilization is one kind of the enzyme modifications by binding or attaching enzymes physically and*	Hard-to-read text	Clarity

	chemically to the supporting solids (immobilizing matrix) like chitosan, carrageenan, zeolite, etc which are insoluble in water.		
34.	~~attaching~~ → connecting, linking	Word Choice	Engagement
35.	~~the~~ solid	Determiner Use (a/an/the/this, etc.)	Correctness
36.	~~solid~~ → reliable	Word Choice	Engagement
37.	*is expected*	Passive Voice Misuse	Clarity
38.	, so	Punctuation in Compound/Complex Sentences	Correctness
39.	A carrageenan	Determiner Use (a/an/the/this, etc.)	Correctness
40.	~~has the ability to~~ → can	Wordy Sentences	Clarity
41.	, which	Punctuation in Compound/Complex Sentences	Correctness
42.	*is expected*	Passive Voice Misuse	Clarity
43.	~~is able to~~ → can	Wordy Sentences	Clarity
44.	The hydrolysis reaction of palm oil observed the effectiveness of immobilization	Passive Voice Misuse	Clarity
45.	~~aquadest~~ → aqua dest	Misspelled Words	Correctness
46.	*monohydrogen*	Unknown Words	Correctness
47.	*The materials used in this study were palm oil, carrageenan, pancreatic lipase enzyme, Whatman 41 filter paper, pH indicator paper, aquadest, n-hexane, ethanol, sodium hydroxide (NaOH), sodium monohydrogen*	Hard-to-read text	Clarity

	phosphate (Na2HPO4.2H2O), sodium dihydrogen phosphate (NaH2PO4.H2O), biuret reagent, indicat...		
48.	~~by means of~~ → using, utilizing, employing, through	Wordy Sentences	Clarity
49.	~~then added~~ → then added	Improper Formatting	Correctness
50.	~~added~~ → to add	Incorrect Verb Forms	Correctness
51.	The formula determines normality of NaOH	Passive Voice Misuse	Clarity
52.	the weight	Determiner Use (a/an/the/this, etc.)	Correctness
53.	0,126 :	Improper Formatting	Correctness
54.	N :	Improper Formatting	Correctness
55.	~~mL~~ → ml	Confused Words	Correctness
56.	mark,	Punctuation in Compound/Complex Sentences	Correctness
57.	~~standard~~ → stock	Word Choice	Engagement
58.	~~standard~~ → traditional, legal	Word Choice	Engagement
59.	*were obtained*	Passive Voice Misuse	Clarity
60.	~~standard~~ → stock	Word Choice	Engagement
61.	the standard	Determiner Use (a/an/the/this, etc.)	Correctness
62.	~~mixture~~ → variety, combination	Word Choice	Engagement
63.	~~aquadest~~ → aqua dest	Misspelled Words	Correctness

64.	the absorbance	Determiner Use (a/an/the/this, etc.)	Correctness
65.	*are used*	Passive Voice Misuse	Clarity
66.	~~sample protein~~ → sample protein	Improper Formatting	Correctness
67.	*is obtained*	Passive Voice Misuse	Clarity
68.	~~standard~~ → ordinary	Word Choice	Engagement
69.	*is put*	Passive Voice Misuse	Clarity
70.	, and	Punctuation in Compound/Complex Sentences	Correctness
71.	~~aquadest~~ → aqua dest	Misspelled Words	Correctness
72.	*was then transferred*	Passive Voice Misuse	Clarity
73.	falcon.	Closing Punctuation	Correctness
74.	~~bottle~~ → Bottle	Improper Formatting	Correctness
75.	*is stirred*	Passive Voice Misuse	Clarity
76.	*To control the reaction*	Misplaced Words or Phrases	Correctness
77.	*is used*	Passive Voice Misuse	Clarity
78.	, then	Punctuation in Compound/Complex Sentences	Correctness
79.	V :	Improper Formatting	Correctness
80.	N :	Improper Formatting	Correctness
81.	~~by means of~~ → using, utilizing, employing, through	Wordy Sentences	Clarity

82.	, then	Punctuation in Compound/Complex Sentences	Correctness
83.	, then	Punctuation in Compound/Complex Sentences	Correctness
84.	*is immobilized*	Passive Voice Misuse	Clarity
85.	, and	Comma Misuse within Clauses	Correctness
86.	~~in accordance with~~ → by, following, per, under	Wordy Sentences	Clarity
87.	*was carried*	Passive Voice Misuse	Clarity
88.	*lipase enzyme activity test method*	Intricate Text	Clarity
89.	*are replaced*	Passive Voice Misuse	Clarity
90.	*was carried*	Passive Voice Misuse	Clarity
91.	~~oC~~ → of	Misspelled Words	Correctness
92.	*is then used*	Passive Voice Misuse	Clarity
93.	, and	Comma Misuse within Clauses	Correctness
94.	*been used*	Passive Voice Misuse	Clarity
95.	*are separated*	Passive Voice Misuse	Clarity
96.	~~concentration~~ → concentrations	Incorrect Noun Number	Correctness
97.	, and	Comma Misuse within Clauses	Correctness
98.	*is then used*	Passive Voice Misuse	Clarity
99.	~~certain~~ → specific	Word Choice	Engagement
100.	~~temperature~~ → weather, climate	Word Choice	Engagement
101.	~~a~~ high	Determiner Use (a/an/the/this,	Correctness

		etc.)	
102.	*were carried*	Passive Voice Misuse	Clarity
103.	~~normal~~ → average	Word Choice	Engagement
104.	the thermal	Determiner Use (a/an/the/this, etc.)	Correctness
105.	*are presented*	Passive Voice Misuse	Clarity
106.	~~Figure~~ → Figures	Incorrect Noun Number	Correctness
107.	~~oC~~ → C	Misspelled Words	Correctness
108.	~~of~~	Wrong or Missing Prepositions	Correctness
109.	*be used*	Passive Voice Misuse	Clarity
110.	~~highest~~ → most increased	Word Choice	Engagement
111.	. This	Punctuation in Compound/Complex Sentences	Correctness
112.	~~temperature~~ → weather	Word Choice	Engagement
113.	*This*	Intricate Text	Clarity
114.	~~shows~~ → indicates	Word Choice	Engagement
115.	temperature,	Punctuation in Compound/Complex Sentences	Correctness
116.	*is damaged*	Passive Voice Misuse	Clarity
117.	~~activity~~ → action, training, move, movement	Word Choice	Engagement
118.	*This*	Intricate Text	Clarity
119.	~~denaturation~~ → Denaturation	Improper Formatting	Correctness

120.	*denaturation due to heat [11].*	Incomplete Sentences	Correctness
121.	*be trapped*	Passive Voice Misuse	Clarity
122.	, which	Punctuation in Compound/Complex Sentences	Correctness
123.	*Immobilization causes lipase to be trapped in carrageenan which protects enzymes from temperature denaturation, so that enzyme conformation is maintained [9].*	Hard-to-read text	Clarity
124.	~~is able to~~ → can	Wordy Sentences	Clarity
125.	*be reused*	Passive Voice Misuse	Clarity
126.	, the	Punctuation in Compound/Complex Sentences	Correctness
127.	the reuse	Determiner Use (a/an/the/this, etc.)	Correctness
128.	~~acssay~~ → assay	Misspelled Words	Correctness
129.	*are presented*	Passive Voice Misuse	Clarity
130.	~~Figure~~ → Figures	Incorrect Noun Number	Correctness
131.	it can	Incomplete Sentences	Correctness
132.	~~is able to~~ → can	Wordy Sentences	Clarity
133.	, as	Punctuation in Compound/Complex Sentences	Correctness
134.	~~in accordance with~~ → by, following, per, under	Wordy Sentences	Clarity
135.	: Erlangga	Improper Formatting	Correctness
136.	: WH	Improper Formatting	Correctness

137.	~~Balcao~~ → Balco, Balcony	Misspelled Words	Correctness
138.	Paiva,	Punctuation in Compound/Complex Sentences	Correctness
139.	: WH	Improper Formatting	Correctness
140.	~~Hydroxil~~ → Hydroxyl	Misspelled Words	Correctness
141.	*OPEN ACCESS Thermal and Reused Stability of Immobilized Lipase in Carrageenan*	Thermal and Reused Stability of Immobilized Lipase in ... https://iopscience.iop.org/article/10.1088/1755-1315/292/1/012028	Originality
142.	*View the article online for updates and enhancements. This content was downloaded from IP address*	A Durable, Inexpensive and Scalable Redox Flow Battery ... https://iopscience.iop.org/article/10.1149/1945-7111/ab84f8/pdf	Originality
143.	*Thermal and Reused Stability of Immobilized Lipase in Carrageenan F A Wardoyo1,*	Thermal and Reused Stability of Immobilized Lipase in ... https://iopscience.iop.org/article/10.1088/1755-1315/292/1/012028	Originality
144.	*Abstract. Lipase is one kind of enzyme that is widely used as a catalyst in industrial and medical. Lipase has a great catalytic ability, but is easily affected by temperature, and is also difficult to separate at the end of the reaction. This causes most of lipase used only in one reaction cycle. ...*	Thermal and Reused Stability of Immobilized Lipase in ... https://iopscience.iop.org/article/10.1088/1755-1315/292/1/012028	Originality
145.	*of the reaction making it difficult to reuse*	WO2018131430A1 - Method for producing organosilicon ... https://patents.google.com/patent/WO2018131430A1/en	Originality
146.	*Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s)*	Validation of VARK learning modalities questionnaire using ... https://iopscience.iop.org/article/10.1088/1742-6596/588/1/012048/pdf	Originality

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