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Identifying Students' Undergraduate Concept Understanding and Misconceptions on Amino Acids, Proteins, and Enzymes Using A Four-Tier Diagnostic Test

Syahmani*, Rusmansyah, Leny, and Rizqa Masila

Chemistry Education, Faculty of Teacher Training and Education
Universitas Lambung Mangkurat, Banjarmasin, Indonesia
*syahmani_kimia@ulm.ac.id

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Abstract

Students' misconceptions have become common in the teaching and learning process, particularly in the challenging subject of biochemistry. This research aims to identify undergraduate students' conceptual understanding and misconceptions of amino acids, proteins, and enzymes. The research method is descriptive qualitative, and data about student conception profiles are retrieved using a four-tier diagnostic test (FTDT). The research subjects comprised 27 participants, including the Chemistry Education Study Program at Universitas Lambung Mangkurat (ULM) Banjarmasin. The sampling technique used in this study was purposive sampling. Data collection with a four-tier diagnostic test instrument consisting of 8 questions, categorizing concept understanding into understanding, not understanding the concept, and misconceptions. Data analysis was conducted by converting the results of the four-tier diagnostic test instrument into percentage data. The results showed that in the amino acids, proteins, and enzymes, of the five concepts tested, 36.67% were in the category of understanding the concept, 15.19% were in the category of not understanding the concept, and 48.15% were in the category of misconceptions. In conclusion, students' concept understanding and misconceptions are moderate. Recommended that innovative learning methods be applied to improve students' understanding of concepts in the sub-material of amino acids, proteins, and enzymes

Keywords: amino acids; enzymes; four-tier test diagnostic; misconceptions; proteins

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INTRODUCTION

Biochemistry is a science that focuses on analyzing the chemical composition of organisms, the structure of their constituent materials, and the metabolism that occurs in the bodies of living creatures (Butnariu et al., 2018; Wahyuni, 2019). The Biochemistry Course aims for students to understand the interconnected theories, principles, concepts, and knowledge they can use in everyday situations (Dewi, 2022).

Our knowledge of students' conceptual grasp of biochemistry has been limited thus far (Villafañe et al., 2011; Halmo et al., 2018; Kurniawati & Jailani, 2020). Teachers also lack effective and trustworthy diagnostic tools to assess students' biochemistry knowledge (Bretz & Linenberger, 2012; Villafañe et al., 2021). Biochemistry and the entire area of life sciences rely heavily on concepts related to proteins, enzymes, and amino acids (Tansey et al., 2013).

Prior research has solely examined college students' conceptual challenges with proteins, enzymes, and amino acids. Students found it most challenging to comprehend the acid-base characteristics of amino acids and the interactions between their non-polar side chains. Understanding all four levels of protein structure was shown to be problematic for students. In contrast, students found it challenging to comprehend enzyme kinetics, enzyme-substrate interactions, and various forms of enzyme inhibition.

Understanding concepts is crucial in building the foundation of students' initial knowledge before entering the learning stage. Students often face difficulties in understanding various chemical concepts. Experts argue that understanding chemical concepts that are not the truth is called a chemical misconception (Izza et al., 2021). Silung et al. (2016) state that this misconception can affect student learning outcomes. If errors occur in understanding a concept, students may not be able to analyze these concepts well, which, in the end, can be detrimental to achieving learning objectives and hurt student learning outcomes and achievements (Azura et al., 2017).

Misconceptions occur because chemical concepts are abstract, requiring complicated abstract reasoning (Nugroho & Prayitno, 2021). Understanding them requires special attention and a high level of focus. These abstract concepts are important because further or theoretical chemistry/science concepts will be difficult to understand if students do not understand the underlying concepts (Fahmi & Irhasyuarna, 2017). Abstract ideas are complicated to know when learning science because understanding them requires a high level of thinking.

One instrument for identifying conceptual understanding and misconceptions experienced by students is the Four Tiers Diagnostic Test (FTDT) instrument (Purwanto et al., 2018; Negoro & Karina, 2019). This four-tier diagnostic test is a two-tier test with two more tiers of self-confidence. Adding these two levels of self-confidence can make identifying misconceptions more accurate. Four analytical answers reflect one indicator of the item under study, which will describe the state of student misconceptions regarding that indicator in more detail.

This study requires developing a four-tier FTDT test to identify students' conceptual knowledge of amino acids, proteins, and enzymes. Prior studies have only examined college students' conceptual difficulties in particular subject areas. Furthermore, none addresses the shortcomings of all previously created diagnostic tools for assessing conceptual knowledge using a four-level exam (Caleon & Subramaniam, 2010).

METHOD

The research method is descriptive qualitative (Creswell, 2015), and to obtain data about students’ conceptual understanding and misconceptions of amino acids, proteins, and enzymes, a four-tier diagnostic test (FTDT). The research subjects comprised 27 undergraduate students in their second year who enlisted in Basic Biochemistry class A2 of the Chemistry Education Study Program, ULM Banjarmasin.

The sampling technique in this study used a purposive sampling technique, and the data collection technique used was a test. The test identified students' misconceptions about amino acids, proteins, and enzymes through comprehension and diagnostic tests supplemented with reasons and beliefs in answering questions according to the simplified four-tier grouping criteria method in Table 1. Such scales were also used in other previously developed four-tier chemistry tests (Sreenivasulu & Subramaniam, 2013, 2014; Yan & Subramaniam, 2018; Habiddin & Page, 2019; Gurel et al., 2015).
Data from student answers is collected and measured in the form of numbers (Hidayah et al., 2018). The numbers obtained are then described in Table 2 according to the level of understanding, lack of knowledge, and misconceptions.

Table 1 Four-tier grouping criteria

Criteria	Answer	Answer Confidence	Reason	Reason Belief
Understand	Right	Tall	Right	Tall
Do not understand	Right	Low	Right	Low
	Right	Tall	Right	Low
	Right	Low	Right	Tall
	Right	Low	Wrong	Low
	Right	Low	Wrong	Low
	Wrong	Low	Wrong	Low
	Right	Tall	Wrong	Low
	Wrong	Low	Right	Tall
Misconceptions	Right	Low	Wrong	Tall
	Right	Tall	Wrong	Tall
	Wrong	Tall	Right	Low
	Wrong	Tall	Right	Tall
	Wrong	Tall	Wrong	Low
	Wrong	Low	Wrong	Tall
	Wrong	Tall	Wrong	Tall

(Gurel et al., 2015)

Table 2 Description of the level of understanding, not understanding the concept and misconceptions

Understanding the concept	Not understanding the concept	Misconceptions	Category
$0\% < P_n \leq 30\%$	$0\% < T_n \leq 30\%$	$0\% < M_n \leq 30\%$	Low
$30\% < P_n \leq 70\%$	$30\% < T_n \leq 70\%$	$30\% < M_n \leq 60\%$	Moderate
$70\% < P_n \leq 100\%$	$70\% < T_n \leq 100\%$	$60\% < M_n \leq 100\%$	High

(Kurniawan & Suhandi, 2015; Sheftyawan et al., 2018)

The percentage level of understanding the concept, not understanding the concept, and misconceptions is calculated using the equation:

$$P_n = \frac{y}{N} \times 100\% \quad , \quad T_n = \frac{z}{N} \times 100\% \quad , \quad M_n = \frac{x}{N} \times 100\%$$

Information:
P_n = Understand the concept of the nth question
y = Many students are categorized as understanding the concept in the nth question
T_n = Do not understand the concept of the nth question
z = Many students are categorized as not understanding the concept in the nth question
Mn = Misconceptions about the nth question
x = Many students are categorized as having misconceptions about the nth question
N = Many students

Analysis of validity and reliability

Instrument validation is carried out to determine the instrument's content validity level. Therefore, the test content should be based on the main topics given to students (Cohen et al., 2017). Five validators carried out the validity test of the eight diagnostic test items. The data analysis technique used in the validation test is Aiken's V formula. All question items were declared suitable for testing understanding of amino acids, proteins, and enzymes. The highest Aiken's score obtained was 0.90, and the lowest was 0.85, which means "valid." This refers to the validity category of the student's conceptual understanding instrument: $85 < x \leq 100$ (very valid), $70 < x \leq 85$ (valid), $50 < x \leq 70$ (less valid), and $x \leq 50$ (invalid) (Yunita et al., 2021). The instrument reliability test results were 0.89 with the "high" category referring to Cronbach's Alpha reliability with the categories: $0.90 < a \leq 1$ (very high), $0.70 < a \leq 0.90$ (high), $0.50 < a \leq 0.70$ (medium), $a \leq 0.50$ (low) (Syahmani et al., 2017).

RESULT AND DISCUSSION

Understanding Student Concepts

Based on research carried out with instruments that are four-tier tests with multiple-choice questions, student answers are grouped into categories of level of concept understanding based on answer patterns, namely understanding, not understanding the concept, and misconceptions of the eight questions tested. After processing the data, it was obtained based on the answer categories in Table 3.

Table 3 Results of concept understanding test

Concepts	Indicators	Question	SK (%)	LK (%)	M(%)
Amino Acid Structure	Find amino acids that react	1	62.96	11.11	25.93
Amino Acid Reactions	Explain the amphoteric nature of amino acids	2	33.33	14.81	51.85
Protein Structure	Relate the structure of amino acids with protein structure Analyzing peptide bonds in proteins in electrophoresis experiments	3 and 4	27.78	9.26	62.96
Factors that influence enzyme activity	Analyze experiments on implementation factors that influence enzyme activity Comparing experiments on implementation factors that influence enzyme activity Analyze experiments on enzymes	5,6, and 8	22.22	18.52	59.26
Enzyme kinetics: Michaelis-Menten equation	Analyze/interpret the graphs of Michaelis Menten	7	37.04	22.22	40.74
Avg			**36.67**	**15.19**	**48.15**

Scientific Concept or Scientific Knowledge (SK), Incomplete Concept or Lack of Knowledge (LK), Misconception (M)

The best level of concept understanding is in the amino acid structure sub-concept, with a percentage of concept understanding in the medium category of 62.96%, and the lowest is in the sub-concept of factors influencing enzymes in the low category with a percentage of 22.22%. Apart from that, the level of understanding of the concept of not understanding the concept is shown by the enzyme kinetics sub-concept, namely with the highest percentage not understanding the concept at 22.22% in the low category and the lowest percentage not understanding the concept, namely in the protein structure sub-concept at 9.26%. Most students' understanding of concepts is also in categories. The highest misconception is protein structure at 62.69%, which is included in the high misconception category, while the lowest misconception is 25.94% in the sub-concept of amino acid structure.

The average understanding of students' concepts in Table 7 shows that most students experienced misconceptions 48.15%, 15.19% did not understand the concept, and the other 36.67% understood the concept. These three categories are divided into small parts: understanding, not understanding the concept, and misconceptions.

There are three sub-concepts at the medium level of conceptual understanding and two at the low category of conceptual understanding. Three sub-concepts in the medium category, namely the highest percentage in the amino acid structure sub-concept 62.96%, the enzyme kinetics sub-concept 37.04%, and the amino acid reaction 33.33%. Two other sub-concepts with a level of concept understanding in the low category are the sub-concept of protein structure, with a percentage of 27.78%, and the sub-concept of factors that influence enzyme activity, with the lowest of 22.22%. Differences in understanding this concept do not mean not understanding; there are also misconceptions, which will be explained in the next section.

Student Misconceptions

Students are not only categorized as understanding or not understanding the concept because they may experience misconceptions about the material they have studied. Table 4 shows the high level of misconceptions that occur in all sub-concepts.

Table 4 Results of students' misconceptions

Concepts	**Question**	**Percentage (%)**	**Category**
Amino Acid Structure	1	25.93	Low
Amino Acid Reactions	2	51.85	Moderate
Protein Structure	3 and 4	62.96	High
Factors that influence enzyme activity	5, 6, and 8	59.26	Moderate
Enzyme kinetics: Michaelis-Menten equation	7	40.74	Moderate

The sub-concept with the highest misconception is the protein structure sub-concept, with a percentage of 62.96% in the high misconception category. The sub-concept with the lowest misconception is the sub-concept of the amino acid structure, with 25.93% in the low misconception category. Meanwhile, other sub-concepts are included in the moderate category of misconceptions, with a percentage of sub-concepts of factors that influence enzymes of 59.26%, followed by the sub-concept of amino acid reactions of 51.85%, and finally, the sub-concept of enzyme kinetics with a percentage of misconceptions of 40.74%.

Analysis of Student Concept Understanding

Difficulties in learning can be indicated by the student's ability to understand concepts and think to solve biochemical problems (Halmo et al., 2018). Misconceptions can occur because students make mistakes in understanding a concept (Barke et al., 2009). Misconceptions are an understanding.

The concepts possessed by students differ from the understanding of concepts established by experts. A student is said to have a misconception if the student can answer one or even none of the questions correctly at the first or second level but is confident in the chosen answer (Hidayati et al., 2019; Syahmani et al., 2023). The existence of misconceptions will make it difficult for students to understand further concepts (Barasbanyu et al., 2021). Mubarak et al. (2016) stated that if the percentage of student misconceptions is more than 10%, then further discussion must be carried out regarding the concepts experiencing misconceptions.

In this study, all concepts had a percentage of misconceptions greater than 10%. This research finding is exciting because all students in this sub-material experience misconceptions. Table 5 presents an explanation regarding understanding concepts, misconceptions, and not understanding concepts experienced by students in more detail.

Table 5 Forms of students' misconceptions

Concepts	**Question**	**Misconceptions**	**Forms of Student Misconceptions**
Amino Acid Structure	1	25.93%	Students assume that amino acids play a role in the test xanthoprotein is not phenylalanine (lysine, serine, cysteine, or glutamate acids) because, in the xanthoprotein test, the base side chains of amino acids react with nitric acid, forming salts.

Concepts	Question	Misconceptions	Forms of Student Misconceptions
Amino Acid Reactions	2	51.85%	Students experience misconceptions about amino acid reactions in determining the protein charge at a certain pH and consider that the most dominant form of glycine at pH 11 is NH_2 -CH_2COOH.
Protein Structure	3,4	62.96%	Glycine is the amino acid least likely to be found at position 10 of the alpha helix amino acid sequence. Smaller polypeptides will bind more quickly to the gel to reach the positive pole of the electric field than larger polypeptides.
Factors that influence enzyme activity	5,6,8	59.26%	The reaction rate increases as the temperature increases. The enzymatic reaction rate in a test tube to which a competitive inhibitor is added will not change, while the enzymatic reaction rate in a test tube to which a non-competitive inhibitor is added will increase. Ammonium sulfate irreversibly reduces the solubility of the enzyme in water, leading to its precipitation, but the enzyme is not denatured.
Enzyme kinetics: Michaelis-Menten equation	7	40.74%	Students interpret the Michaelis-Menten graph in that the graph's peak depicts the Vmax of the enzyme reaction at the optimum temperature.

Amino Acid Structure

In the sub-concept of amino acid structure, the majority of students have a good understanding of the concept, and this is shown by the level of concept understanding of 62.96%, which is the highest percentage compared to other sub-concepts. Students in this condition must have mastered the material or concepts provided well (Abraham et al., 1992). They can differentiate between what they know and what they do not know. However, 25.93% of other students still experience misconceptions, and 11.11% do not understand the concepts, as in Figure 1.

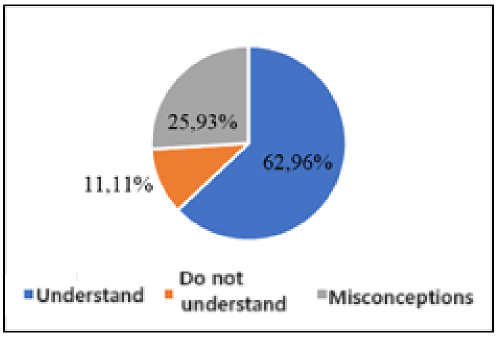

Figure 1 Results of understanding of amino acids structure

In the FTDT questions in this sub-concept, several answer choices are presented: lysine, serine, cysteine, phenylalanine, and glutamic acid; then, students choose the amino acids. After being heated with nitric acid, it plays a role in the xanthoprotein test. Most of the students' answers were phenylalanine because phenylalanine undergoes nitration when heated in nitrate. However, other misconceptions vary, where students assume that what plays a role in the xanthoproteins test when heated with nitrate is not phenylalanine but lysine, serine, cysteine, or glutamic acid.

Apart from errors in the first level, students also experience misconceptions about the reasons for choosing this answer. Students' answers are spread across two options, namely (1) in the xanthoproteins test, the base side chain of the selected amino acid reacts with nitric acid, forming a salt (2) In the xanthoproteins test, the sulfhydryl group of the side chain of the selected amino acid undergoes oxidation, with nitric acid which acts as an oxidizing agent (Mulyani & Murkhozin, 2017). Meanwhile, the correct concept is that the aromatic side chain of the amino acid chosen in this problem, namely phenylalanine, undergoes nitration. According to Sadler and Sonnert (2016), teachers could previously only identify 42.7% of students as having alternative conceptions about xanthoprotein reactions, and the teacher noted that none of the reactions presented problems for the students, indicating that this kind refers to the reaction of amino acids.

Amino Acid Reactions

As shown in Figure 2, the sub-concept of amino acid reactions experienced 51.85% misconceptions in the "medium" category, 14.81% did not understand the concept, and 33.33% understood it.

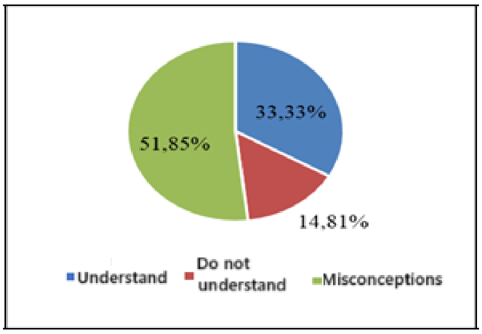

Figure 2 Results of understanding of amino acid reactions

Questions in the sub-concept present about glycine's structure and net charge at pH 11. The tests students' conceptual understanding of the amino acid reaction process. In the lessons, the lecturer provides examples of reactions related to amino acid reactions. However, students' ability to recognize and understand questions is still low, so it is difficult for them to answer questions different from the examples given by the lecturer (Caleon & Subramaniam, 2010). In this question, students assume that the most dominant form of glycine at pH11 is $NH_2 – CH_2COOH$. This is a misconception because the most dominant form of glycine at pH 11 is $NH_2 – CH_2COO^-$.

Structure protein

The results of students' conceptual understanding of the protein structure sub-concept are presented in Figure 3, which shows that in the protein structure sub-concept,t students experienced the highest misconceptions with a percentage of 62.96% in the "high" category, 27.78% of students understood the concept, and nine students did not understand the concept 9.26%.

The form of student misconception in this sub-concept is that the amino acid that is least likely to be found in position 10 of the alpha helix amino acid sequence is proline for the reason that it assumes that the side chain of proline is too large to fit inside the alpha helix or believes that the proline side chain cannot take part. in the formation of hydrogen bonds which are essential for the stability of the alpha helix.

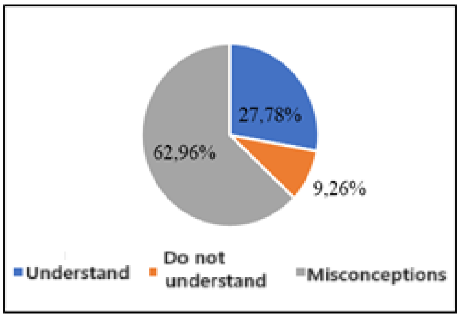

Figure 3 Results of understanding of protein structure

The explanation above states that students answered correctly that the amino acid proline is the amino acid least likely to be found in the 10th position in the alpha helix of amino acids. However, students gave the wrong reasons. The exact cause is because of the structure of proline where after peptide bond formation occurs, the α-amino group of proline cannot form a hydrogen bond with the oxygen atom of the amino acid carbonyl group at position 6, and this type of hydrogen bond is essential for the stability of the alpha helix. In addition, rotation of the N-Cα bond in proline is not possible, which causes instability in the helical structure. The question of the sub-concept of protein structure is quite difficult because it is related to the structure of a compound. The question of compound structure in the submicroscopic and symbolic depiction. This depiction is abstract and cannot be experienced by students in everyday life (Dewi *et al*., 2018; Halmo et al., 2018).

The percentage of students who experience misconceptions about the structure of proteins is greater than the percentage of misconceptions about the structure of amino acids. Even though both are or are formed by amino acids, the protein structure has a structure and bonds that can stabilize it, which are more complex than the structure of amino acids. An alternative solution from Harle & Towns (2013) states that students need to understand the construct of primary and secondary protein structures by drawing secondary protein structures. This reveals student understanding better than simple recognition of structures.

Factors influencing enzyme activity

The misconception in this sub-concept is that enzymes have an optimal temperature of 20°C. When the system temperature is increased to 37.5°C, the rate of enzymatic reactions will significantly increase by collision theory; namely, the rate of all reactions increases as the temperature increases. This misconception occurs because it does not consider why enzymes are proteins, where an increased temperature beyond the optimum temperature can cause a denaturation process. The active part of the enzyme will be disturbed; thus, the enzyme's effective concentration will be reduced, and the reaction speed will decrease. For different questions still in the sub-concept, misconceptions about the factors influencing the enzyme also occur. The misconception is that students answer that the rate of enzymatic reactions in test tubes where inhibitors are added is non-competitive when the substrate concentration is increased, the non-competitive inhibitor will bind to the allosteric site of the enzyme, after which the binding of the substrate to the active site is greatly inhibited. However, more molecules will successfully bind to the active site as the substrate increases. This shows that students do not understand that this non-competitive inhibitor will bind to the allosteric site, have the same affinity for the enzyme and enzyme complex, and not affect the binding of the substrate to the active site. However, this non-competitive inhibitor will reduce the enzyme's catalytic activity after successfully binding the enzyme on the allosteric side.

The questions in this sub-concept are application or application questions. Most students have difficulty with questions related to the application. Students understand the concept only partially, without delving into it, so they are confused when choosing answers. Students connect concepts with their thought patterns (Prihatni *et al*., 2016).

The interpretation of students' conceptual understanding of the factors that influence enzymes is shown in Figure 4. In the diagram, the level of misconception of this sub-concept is 59.26%. This is because most students answer incorrectly at the level of giving reasons. An answer pattern like this can be said to be a positive misconception, also known as a *false positive*, where the condition of the response given by the student is correct in the content of the concept being asked but cannot provide the right scientific reasons to strengthen the idea he has (Mubarak *et al*., 2016).

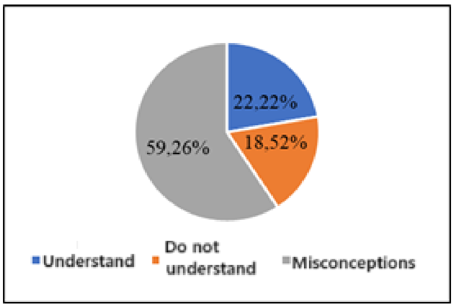

Figure 4 Results of understanding of factors that influence enzymes

Enzyme Kinetics: The Michaelis-Menten Equation

Figure 5 presents students' conceptual understanding of this sub-concept. The majority are misconceptions, namely 40.74%, while other students are at the level of understanding the concept at 37.04% and do not understand the concept at 22.22%.

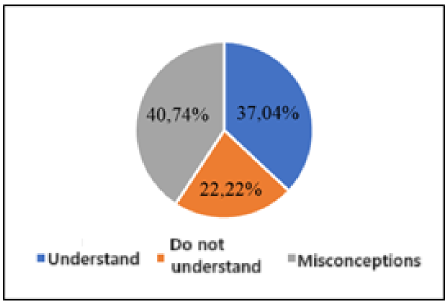

Figure 5 Results of understanding of enzyme kinetics

In the question, students are asked to interpret the presented Michaelis-Menten graph. The misconception experienced by students is that the peak of the Michaelis-Menten graph depicts the Vmax of the enzyme reaction at the optimum temperature. This is different from Michaelis and Menten's statement that the reaction speed depends on the concentration of the enzyme-substrate complex (ES), because if it depends on the concentration of the substrate (s), then the increase in reaction speed, when depicted, will be a straight line (Mulyanti & Murkhozim, 2017; Rodrigez et al., 2019; Rodriguez & Towns, 2021).

Misconceptions can arise because students have difficulty assimilating new concepts, so they are mixed with experience (Suwarto, 2013). Misconceptions can also occur when the learning process is not comprehensive, which makes students build knowledge by understanding their concepts (Roghdah, Zammi, & Mardhiya, 2021).

Based on the analysis carried out, it is necessary to improve the quality of learning so that students can develop an understanding of fundamental chemical concepts by learning carried out using a multi-representation approach to chemistry, which involves all representations starting from the macroscopic level, sub-macroscopic level and symbolic (Becker et al., 2015). A multi-level approach to representation and metacognition can be integrated into chemistry learning to increase understanding of concepts and, at the same time, reduce chemical misconceptions (Avigril, 2019; Suparwati, 2022).

CONCLUSION

The level of student conceptual understanding is 36.67% in the "medium" category, with the highest level of conceptual knowledge in the structure of amino acids, namely 62.96% in the "medium" category, and the lowest level of conceptual understanding in the factors that influence enzymes, namely 22 .22% in the "low" category. Apart from that, the students' misconception level was 48.15% in the "medium" category, with the highest level of misconception in the protein structure, namely 62.96% in the "high" category, and the lowest level of misconception in the amino acid structure, namely 25.93% in the "high" category "low." It is recommended that innovative learning methods be applied to improve the quality of learning and increase students' conceptual understanding of proteins and enzymes.

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