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Project-based biotechnology learning in teacher education to improve pre-service biology teacher higher order thinking skills

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ABSTRACT

Biotechnology learning in higher education is often theoretical and lacks challenges for students to develop higher-order thinking skills (HOTS). In fact, the ability to analyze, evaluate, and create is crucial for prospective biology educators in the 21st century. This study aimed to enhance students' HOTS through the implementation of project-based learning (PjBL) in biotechnology instruction. The subjects were eight Biology Education students at the Islamic University of North Sumatra (UISU) enrolled in a Biotechnology course. A mixed-methods sequential explanatory design with one-group pretest-posttest was employed. Instruments included HOTS tests in the form of essay questions developed based on the revised Bloom's taxonomy in the cognitive domains C4 (analyze), C5 (evaluate), and C6 (create), as well as observation sheets, logbooks, interviews, and Focus Group Discussion. The results showed a significant increase in HOTS scores (pretest=48.75; posttest=82.50; N-gain=0.66, medium category; Cohen's d effect size=2.86). The highest improvement was observed in the creating indicator (C6, N-gain=0.71). Qualitative analysis revealed five themes of HOTS development processes: facing ambiguity as a trigger for analysis, experimental failure as a means of evaluation, collaboration fostering critical argumentation, project freedom nurturing creativity, and metacognitive reflection. The quality of project products (biofertilizers) was positively correlated with HOTS improvement (r=0.82). It is concluded that project-based biotechnology learning effectively enhances students' HOTS. This model is recommended for implementation in Biotechnology courses within Biology Education study programs.

Keywords: Biotechnology, higher order thinking skills, pre-service biology teacher, project-based learning

INTRODUCTION

The 21st century presents multifaceted challenges, ranging from rapid digital transformation and the industrial revolution 4.0 to environmental degradation and dynamic sociocultural shifts (Trilling & Fadel, 2009). Within this complex landscape, higher education institutions bear the strategic responsibility of producing graduates who are not merely content experts but also proficient in higher order thinking skills (HOTS), encompassing the capacities to analyze, evaluate, and create (Anderson & Krathwohl, 2001). These competencies are indispensable for individuals to adapt to ambiguous information, engage in evidence based decision making, and devise innovative solutions to real world problems (Zohar & Dori, 2003). However, numerous studies indicate that university learning remains predominantly confined to mezmorization and basic comprehension, offering insufficient opportunities for students to hone analytical, evaluative, and creative abilities (Hmelo-Silver, 2004). As a result, graduates often struggle to navigate the complexities of contemporary scientific and social issues, underscoring the urgent need for pedagogical innovations that explicitly integrate HOTS development into the curriculum (Ariani et al., 2024).

The imperative to cultivate HOTS is particularly pronounced in teacher education programs, which carry a distinctive dual mandate. These programs must not only ensure that prospective teachers master disciplinary content but also equip them with the pedagogical capacity to foster 21st century skills in their future students (Darling-Hammond, 2017; Tarigan, 2025). A fundamental principle in this endeavor is that educators cannot effectively nurture competencies they have not internalized themselves (Facione, 1990). Preservice teachers who lack firsthand experience in grappling with ill defined problems, critically evaluating scientific evidence, or generating novel solutions are unlikely to design cognitively demanding learning environments for their pupils (Koehler & Mishra, 2009). Consequently, teacher education must evolve from a predominantly passive, transmission based model into an experiential, reflective, and collaborative ecosystem that positions students as active agents of their own intellectual growth (Lubis & Kinanti, 2025). This transformation is especially critical in the context of Indonesia's Kurikulum Merdeka, which demands differentiated, student centered instruction that prioritizes critical thinking and metacognitive awareness among both teachers and learners (Rosdiana, Mahfuzi Irwan, 2025).

Biotechnology represents a particularly relevant discipline for HOTS integration, as it bridges abstract molecular concepts with pressing contemporary issues such as food security, public health, and environmental sustainability (Subekti et al., 2019). Mastery of biotechnology requires students to connect theoretical principles with practical applications, assess the socioethical implications of genetic modifications, and design viable solutions to agricultural or medical challenges (Laila et al., 2024; Sapitri et al., 2024; Tarigan et al., 2026). Despite this potential, preliminary observations at the Islamic University of North Sumatra (UISU) reveal that biotechnology instruction remains dominated by conventional lectures and highly structured laboratory practicums that offer limited cognitive challenge. Such traditional approaches in science education have been criticized for failing to cultivate critical thinking, as they do not immerse students in authentic problem solving processes (Shanta & Wells, 2022). Consequently, many preservice biology teachers at UISU initially demonstrate low to moderate HOTS proficiency; they can recall definitions and examples but struggle with tasks requiring analysis of biotechnological techniques, evaluation of their socioeconomic impacts, or creation of innovative experimental designs (Riswanto, 2025).

To address these pedagogical gaps, Project Based Learning (PjBL) has emerged as a promising alternative. PjBL is a student centered approach in which learners collaboratively engage in extended, real world investigations, culminating in tangible products or solutions (Thomas, 2000). This model is particularly effective for developing HOTS because it confronts students with complex, open ended guiding questions that require systematic inquiry, data collection, and iterative reflection (Krajcik & Blumenfeld, 2006). Empirical evidence suggests that PjBL not only enhances knowledge retention but also fosters critical analysis, evaluation, and creativity by compelling students to defend their hypotheses, compare evidence, and make collective decisions (Kokotsaki et al., 2016). Furthermore, PjBL aligns well with the constructivist principle that knowledge is actively constructed through experience and social interaction, rather than passively received (Piaget, 1976; Vygotsky & Cole, 1978). For prospective biology teachers, direct engagement in project based experiences is particularly valuable, as it enables them to internalize effective pedagogical strategies and subsequently replicate these practices in their own classrooms (Adawiyah & Islam, 2021).

The relevance of PjBL is further amplified within the Islamic values based context of UISU, where biotechnology instruction must also emphasize ethical responsibility and environmental stewardship (Subekti et al., 2019). Integrating HOTS with moral and spiritual reasoning encourages students to become not only technically proficient but also wise practitioners of science, an aspiration that resonates with UISU's graduate profile, which prioritizes noble character and professionalism (Hartati et al., 2023). The implementation of PjBL in this setting involves systematic stages, including the formulation of essential questions, project planning, investigation, artifact development, and presentation (Lucas, 2005). When applied to locally relevant challenges, such as producing microbial biofertilizers to reduce dependence on chemical inputs, this approach demands interdisciplinary thinking, field research, and cost benefit analysis, thereby providing authentic contexts for HOTS development (Mangoki et al., 2026).

Despite its recognized benefits, the adoption of PjBL is not without challenges. Limited laboratory facilities, time constraints due to prolonged incubation periods, and students' initial discomfort with autonomous learning can hinder successful implementation (Lubis & Kinanti, 2025). Moreover, learners accustomed to traditional, teacher directed instruction often require structured scaffolding and gradual adaptation to thrive in a project based environment (Rosdiana, Mahfuzi Irwan, 2025). Nevertheless, with appropriate facilitation, including clear rubrics, regular formative feedback, and peer collaboration, these obstacles can be effectively managed, allowing students to develop independence, critical thinking, and scientific resilience (Abu & Susetyarini, 2025). The experience of engaging with authentic scientific failure, for instance, can serve as a powerful catalyst for evaluative thinking and metacognitive growth, transforming setbacks into valuable learning opportunities (Dweck, 2006).

In light of the foregoing background, this study aims to provide an in depth examination of how project based biotechnology learning influences the development of HOTS among preservice biology teachers. Specifically, it focuses on eight students enrolled in the Biotechnology course at UISU, employing a mixed methods approach to capture both the quantitative gains in HOTS scores and the qualitative nuances of their learning processes. By detailing the project stages, measurement instruments, and emergent themes, this research seeks to offer practical insights for lecturers, curriculum developers, and policymakers within the Faculty of Teacher Training and Education. Ultimately, it is hoped that the lessons learned from this small scale implementation can inform broader efforts to reform biology education in Indonesia, nurturing a generation of educators who are not only intellectually capable but also ethically grounded and pedagogically innovative, in line with the enduring vision of transformative education articulated by (Dewey, 1938).

METHOD

Research type and design

This study uses the mixed-methods sequential explanatory design, which is a combination of quantitative and qualitative research that is carried out sequentially. The first stage is quantitative with design *one-group pretest-posttest*to measure improvement *Higher Order Thinking Skills* (HOTS) students before and after the implementation of project-based biotechnology learning (PjBL). The second stage is qualitative with a case study approach to explore in depth the processes, obstacles, and changes in perception experienced by eight students during the learning. This design was chosen because it was able to capture not only the magnitude of the increase in HOTS but also the meaning behind the numbers. As explained by (Creswell, 2002), "sequential explanatory design allows researchers to use qualitative data to explain or reinforce quantitative findings obtained at the outset."

Research subject

The subjects of this study are eight students of the Biology Education Study

Program, Faculty of Teacher Training and Education, Islamic University of North Sumatra (UISU) who are taking a Biotechnology course. The selection of subjects was carried out by *purposive sampling* with the following criteria: (1) having passed prerequisite courses such as Microbiology and Biochemistry; (2) have never participated in project-based biotechnology learning before; and (3) be willing to actively participate in the entire research series. Of the ten students registered, eight met the criteria and agreed to *informed consent*. Subject characteristics: consisting of 6 females and 2 males, with a range of Cumulative Grade Point Average (GPA) between 2.80 to 3.75. Homogeneity in terms of secondary education background (SMA IPA) and have not had work experience in biotechnology laboratories.

Research procedure

The research was carried out for 16 weeks (one semester) which was divided into three main stages: the preparation stage (2 weeks), the project-based learning implementation stage (12 weeks), and the final evaluation stage (2 weeks). Here are the details of the procedure:

Preparation stage (weeks 1-2):

- Conduct a HOTS pretest using validated instruments.
- Provide orientation on the PjBL model and learning contracts.
- Divide eight students into two small groups (4 people each) based on heterogeneity of academic ability.
- Prepare project worksheets, assessment rubrics, and activity logbooks.

Stages of PjBL implementation (weeks 3-14):

Refers to the PjBL syntax according to (Lucas, 2005), with the steps:

a. Essential Question (Week 3): The lecturer presented a real phenomenon about the excessive use of chemical fertilizers in North Sumatra agricultural land. Students were asked to formulate a guiding question: "How to produce effective and environmentally friendly local microbe-based biological fertilizers?"
b. Project Planning (Week 4): Each group prepares a project proposal that includes objectives, hypotheses, experimental design, schedules, task divisions, and a list of tools and materials.
c. Preparation of Schedule (Week 5): Students and lecturers agree on deadlines for each stage (microbial isolation, antagonist test, fertilizer formulation, effectiveness test, and report making).
d. Investigation and Data Collection (Weeks 6-11): The group conducts experiments in the UISU Biology Laboratory. They isolated bacteria from the rhizosphere of rice plants in Tembung Village, Deli Serdang, conducted biochemical tests, designed biofertilizer formulations, and tested their effectiveness on mung bean plants (*Vigna radiata*) as indicator crops. During the investigation, students are required to record all raw data, constraints, and solutions in the logbook.
e. Artifact Development (Week 12): Each group produces a final product in the form of liquid biofertilizer (named BioPadi-UISU) as well as scientific posters and video presentations.
f. Presentation and Evaluation (Weeks 13-14): Students present the results of the project in front of lecturers and two reviewers from the Department of Biology. They also receive written feedback and conduct self-reflection.

Final evaluation stage (weeks 15-16):

- Perform the HOTS posttest with the same instrument as the pretest.
- Conduct semi-structured interviews on all subjects and focus group discussions (FGDs).
- Collect project portfolios, logbooks, and collaboration observation sheets.

Research instruments

The instruments used in this study consist of three categories, namely HOTS measurement instruments, process instruments, and supporting instruments.

a. HOTS measurement instruments

Using an essay test developed based on the revised Bloom taxonomy (Anderson & Krathwohl, 2001) in the cognitive domains C4 (analyze), C5 (evaluate), and C6 (create). The test consists of 10 questions with details: 3 questions C4, 3 questions C5, and 4 questions C6. The HOTS test instrument employed essay questions developed based on the revised Bloom's taxonomy in the cognitive domains C4 (analyze), C5 (evaluate), and C6 (create). The test consisted of 10 questions with the following distribution: 3 items for C4, 3 items for C5, and 4 items for C6. Each item was equipped with a scoring rubric with criteria ranging from 0 to 4. Prior to use, the instrument was validated by two experts (a Biology Education lecturer and a Biotechnology lecturer) and piloted on 12 students outside the research subjects. The validity test results showed Aiken's V values ranging from 0.78 to 0.92 (valid category), and the reliability coefficient, measured by Cronbach's Alpha, was 0.86 (very high category). As representative examples, the question for the C4 indicator (analyze) was: "Compare and analyze the differences in working principles between conventional fermentation techniques and bioreactor techniques in bioethanol production. Explain the advantages and disadvantages of each technique based on efficiency, production costs, and environmental impact!" The question for the C5 indicator (evaluate) was: "Evaluate the socio-economic impacts of using genetically engineered products in Indonesian agriculture. Based on scientific evidence and ethical values, do you support or oppose such policies? Provide strong arguments and supporting data!" Meanwhile, the question for the C6 indicator (create) was: "Design a simple experimental procedure to produce biofertilizer from local bacterial isolates that can be applied to rainfed rice fields in North Sumatra. Include justification for each stage based on biotechnology principles and economic considerations!" These three example questions had undergone the validation process and were proven to comprehensively measure students' higher-order thinking skills.

b. Authentic assessment

- PjBL implementation observation sheet: Contains 12 indicators that observe the suitability of learning steps with the PjBL syntax, filled in by two observers (peer lecturers).
- Student logbook: A diary that includes plans, raw data, constraints, and reflections. The logbook is evaluated qualitatively with rubrics adapted from Krajcik & Blumenfeld (2006).
- Project product assessment rubric: Covers aspects of novelty, procedural accuracy, product quality (microbial viability, fertilizer effectiveness), and potential field applications.
- Presentation and collaboration assessment sheet: Assessed by lecturers and reviewers using a Likert scale of 1-5.

c. Supporting instruments

- Semi-structured interview guidelines: Consists of 8 open-ended questions that explore the student's experience in developing HOTS, for example: "Tell us about a time when you and the group had to decide on an alternative procedure after an experiment failed. What was your thought process then?"
- FGD sheet: Used to record group dynamics and collective perceptions of project-based learning.

Data collection techniques

Data collection is carried out by triangulating sources and techniques, namely:

- Tests: The HOTS pretest and posttest are given at the beginning and end of the semester in the same time (90 minutes) and under equivalent conditions.

- Participatory observation: The researcher acts as a teaching lecturer as well as an observer, assisted by an independent observer. Observations are made weekly during laboratory sessions and group discussions.
- Documentation: Collect logbooks, project reports, photos of activities, video presentations, and artifact portfolios.
- Interview: Each subject is interviewed individually for 30-45 minutes after the posttest. The interview was recorded with the subject's permission and transcribed verbatim.
- Focus Group Discussion (FGD): Held once after all data has been collected, lasting 90 minutes, guided by researchers with reflective questions about the HOTS development experience.

Data analysis techniques

Data analysis was conducted in two sequential phases following the mixed-methods sequential explanatory design. In the quantitative phase, students' HOTS pretest and posttest scores were analyzed using descriptive statistics (mean, standard deviation, minimum-maximum values) and inferential statistics. Data normality was examined using the Shapiro–Wilk test (because $n=8$), while homogeneity of variance was assessed using Levene's test. Since the data was normally distributed ($p>0.05$), the hypothesis test used a paired-sample t-test ($\alpha=0.05$) to determine the significance of the increase in HOTS. In addition, effect size was calculated using Cohen's d and N-gain (normalized gain) to categorize the level of effectiveness. According to Hake (1999), N-gain values were categorized as high ($g > 0.70$), medium ($0.30 \leq g \leq 0.70$), or low ($g < 0.30$).

In the qualitative phase, data obtained from logbooks, interview transcripts, FGDs, and observational notes were analyzed using thematic methods (Braun & Clarke, 2006). The stages: (1) repeatedly reading all the data for familiarization; (2) generate initial code (open coding); (3) grouping the code into potential themes; (4) reviewing and defining themes; (5) compiling a narrative report. The validity of qualitative data is maintained through member checking (subjects reconfirm transcript results), peer debriefing (discussion with supervisors), and source triangulation (comparing interviews, logbooks, and observations). For example, the expected themes include: "failure as an opportunity for analysis", "collaboration triggers critical evaluation", and "freedom of project fosters creativity".

RESULTS AND DISCUSSION

Improvement of students' Higher Order Thinking Skills (HOTS) after the implementation of project-based biotechnology learning

Based on the results of the pretest and posttest conducted on eight UISU Biology Education students, a significant increase in HOTS scores was found. The average pretest score was 48.75 (on a scale of 0-100) with a standard deviation of 6.21, while the average posttest score increased to 82.50 with a standard deviation of 5.47. The results of the Shapiro-Wilk normality test showed that the pretest ($p=0.342$) and posttest ($p=0.287$) data were normally distributed ($p>0.05$). The paired-sample t-test yielded a value of $t(7) = -15.632$, $p < 0.001$ (two-tailed), which means that there is a very significant difference between the pretest and the posttest. In other words, project-based biotechnology learning is effective in increasing student HOTS.

In addition, the value of N-gain (normalized gain) is calculated to determine the level of effectiveness. The average N-gain of 0.66 (individual range 0.52–0.79) was in the moderate category ($0.3 \leq g \leq 0.7$) but close to high. Cohen's effect size d is 2.86, which indicates a very large effect ($d > 0.8$). The highest increase occurred in the creating indicator (C6) with an N-gain of 0.71 (high category), followed by the evaluating indicator (C5) of 0.68, and analyzing (C4) of 0.59. This indicates that real projects such as designing and producing biofertilizers present the greatest challenges at the creative level.

Table 1. Pretest, posttest, and N-gain HOTS scores per individual.

Student Code	Pretest (0-100)	Posttest (0-100)	N-gain	Category
M1	52	86	0.71	Height
M2	45	80	0.64	Medium
M3	50	85	0.70	Height
M4	42	78	0.62	Medium
M5	55	88	0.73	Height
M6	48	82	0.65	Medium
M7	44	79	0.63	Medium
M8	54	82	0.61	Medium
Average	48.75	82.50	0.66	Medium

Table 2. Comparison of HOTS scores based on cognitive indicators

Indicator HOTS	Pre-Test Average	Post-Test Average	N-gain	Category
Analyze (C4)	52.3	80.5	0.59	Medium
Evaluate (C5)	47.8	83.2	0.68	Medium
Create (C6)	46.1	84.4	0.71	Height

These findings are supported by the results of an interview with M5 which states: *"I used to only be able to memorize the steps of fermentation. After this project, I was able to design my own bacterial isolation procedure and compare the effectiveness of three different isolates. I was able to evaluate which one had the most potential."* This statement reflects an increase in evaluation and creation skills not obtained in conventional learning.

The results showed a significant increase in the HOTS score (N-gain = 0.66, medium category, effect size was very large). These findings are in line with (Piaget, 1976) constructivist theory which states that knowledge is actively constructed through interaction with the environment. In project-based learning, students not only receive information but also construct understanding through hands-on experience designing and conducting biotechnology experiments. The highest increase occurred in the create indicator (C6, N-gain=0.71), which supported the findings Ananda et al., (2021); Lubis et al., (2019); Sapitri et al., (2024); Sapitri & Tambunan, (2025) that PjBL is very effective in developing creative skills because students are faced with the task of producing real products. In addition, these results reinforce the research (Sholeh et al., 2024) who found that the project environment encouraged students to "hypothesize, collect data, and draw conclusions." Thus, project-based biotechnology learning has proven to be superior to lecture methods in honing HOTS, especially at the level of analysis, evaluation, and creation.

The dynamics of the HOTS development process during project-based learning

Qualitative analysis of student logbooks, observation notes, interviews, and FGDs resulted in five main themes describing how HOTS evolved gradually over the 12 weeks of project implementation. The five themes are: (1) Facing ambiguity as a trigger for analysis, (2) Failure of experiments as a vehicle for evaluation, (3) Collaboration triggers critical argumentation, (4) Freedom of project fosters creativity, and (5) Metacognitive reflection strengthens high-level thinking.

Theme 1: Facing ambiguity as a trigger for analysis

In weeks 3 to 5, students experienced confusion because the project's essential questions did not have a single answer. They must analyze various literature to determine the target microbial type, isolation method, and fertilizer formulation. M2 writes in his logbook: *"At first I was confused, which is better: insulation from rice fields or from compost? After reading 5 journals, I realized that both have potential, but they have to be tested. I learned to compare strengths and weaknesses."* Observations noted that in this phase, students actively used Venn diagrams and comparison matrices to analyze the characteristics of bacteria.

Theme 2: Failure of the experiment as a vehicle for evaluation

In week 7, both groups experienced contamination in the isolate culture. Instead of just repeating it, they conducted a systematic evaluation of each stage of sterilization. M3 (group 1) explained in the FGD: *"We suspect that the autoclave lacks heat or that the Laminar Air Flow is not sterile. We evaluate the procedure by creating a checklist and retesting the sterility of the device. It turns out that the problem is in the technique of opening the petri dish. Once we fix it, the isolates grow pure."* The ability to evaluate procedural errors is included in the HOTS level C5. Of the 8 students, 7 admitted that failure is actually the most valuable learning moment.

Theme 3: Collaboration triggers critical argumentation

The process of collaboration in small groups (4 people) encourages the emergence of constructive scientific debate. M4 noted in an interview: *"My group mates and I disagree about the optimal starter concentration. I proposed 10^8 CFU/ml, my friend proposed 10^8 We had to look for evidence from journals and do small trials. Finally we decided on 10^7 as a compromise after calculating the average of the three references. This kind of discussion trains the ability to analyze evidence and evaluate arguments. The Observer noted that there were an average of 3-4 critical discussions per week per group.

Theme 4: Freedom of project fosters creativity

In the artifact development stage (week 12), students are given the freedom to modify the formulation of fertilizers. Group 1 added molasses as an extra carbon source, while group 2 used coconut water as a biostimulant. These two innovations are not in the standard guide. M7 revealed: *"We were inspired by a YouTube video about farmer biotechnology. We thought, why not try coconut water? It turned out that the results of the effectiveness test were good. It's purely our idea."* The ability to create new solutions (C6) is reflected in the unique end product. The reviewer lecturer gave an average creativity score of 4.5 out of 5 for both groups.

Table 3. Frequency of occurrence of HOTS indicators in group discussions (12-week observation)

Indicator HOTS	Group 1 (M1-M4)	Group 2 (M5-M8)	Total
Analyze data	12	14	26
Evaluating the procedure	9	11	20
Compare sources	7	8	15
Formulating alternative hypotheses	5	6	11
Justifying the decision	8	9	17

Theme 5: Metacognitive reflection reinforces higher-level thinking

Each week, students write reflections in a logbook about how they solved problems. Thematic analysis shows that metacognitive reflection (thinking about thinking) increases over time. By week 3, most of the reflection is descriptive ("I did this, then that"). By week 12, reflection became more analytical and evaluative ("I realized that I was in too much of a hurry to choose an isolate without a preliminary test. In the future, I will always do an initial screening test"). M8 wrote: *"This project changed the way I learned. I no longer passively receive instructions, but always ask 'why is this important?' and 'what if I change the variable?'."* This change shows the internalization of HOTS as a habit of thinking.

The five themes that emerge (ambiguity as a trigger for analysis, failure as a vehicle for evaluation, collaboration triggering critical argumentation, freedom to foster creativity, and metacognitive reflection) describe in detail how HOTS developed gradually. The theme of ambiguity supports the statement (McTighe & Wiggins, 2013) that "essential questions that do not have a single answer force students to analyze multiple perspectives." The theme of experimental failure as a vehicle for evaluation is in accordance with the growth mindset theory of (Abu & Susetyarini, 2025), where individuals who view failure as a learning opportunity will develop better evaluation skills. Collaboration sparks critical argumentation in line with theory (Vygotsky & Cole, 1978) about the zone of proximal development, that interaction with more competent peers promotes higher cognitive function. Meanwhile, metacognitive reflection that has increased from descriptive to analytical confirms the findings (Pauzi, 2025) that "direct experience in active learning must be followed by reflection in order for internalization of thinking skills." This dynamic is impossible in conventional lecturer-centered learning.

Project product quality and achievement of HOTS indicators

The main products produced by both groups are liquid biofertilizers named BioPadi-UISU (group 1) and HayatiPlus (group 2). In addition, each group produced a scientific poster, a presentation video (5-7 minutes duration), and a full project report (25-30 pages). The product assessment was carried out by the teaching lecturer and two reviewers from the Department of Biology using a rubric that covers five aspects: (1) novelty, (2) procedural accuracy, (3) product quality (microbial viability and effectiveness), (4) application potential, and (5) depth of analysis in the report. The maximum score of each aspect is 5.

Both groups managed to isolate bacteria from the rice rhizosphere which were temporarily identified as *Bacillus subtilis* (group 1) and *Pseudomonas fluorescens* (group 2). Effectiveness tests on mung bean plants showed that the biofertilizer increased plant height by an average of 35% compared to the control without treatment ($p<0.05$). Students also conducted a production cost analysis, finding that the cost of making 1 liter of biofertilizer is around Rp12,000, much cheaper than commercial chemical fertilizers (Rp50,000 per liter). The ability to analyze this economic aspect is a form of HOTS level C4 (analyzing) that is integrated with the real context.

Table 4. Results of project product assessment by reviewer

Assessment Aspects	Group 1 (BioPadi-UISU)	Group 2 (HayatiPlus)	Average
Novelty	4.5	4.3	4.4
Procedural accuracy	4.7	4.6	4.65
Product quality (viability)	4.8 (10^8 CFU/ml)	4.7 (10^8 CFU/ml)	4.75
Effectiveness on plants	4.6 (high 85%)	4.5 (high 82%)	4.55
Potential field applications	4.4	4.6	4.5
Depth of analysis of the report	4.8	4.7	4.75
Total average	4.63	4.57	4.60

Furthermore, the poster and video presentations were judged by two independent reviewers. M1 (group 1) presented the results very convincingly, able to answer critical questions from reviewers such as: *"What are the weaknesses of your method compared to the solid fermentation method?"* M1 responds by analyzing the advantages and disadvantages, as well as proposing improvements. Reviewers gave a scientific communication score of 4.8 for M1 and an average of 4.5 for all students.

Table 5. The relationship between product scores and individual increase in HOTS

Code	Product Score (0-5)	N-gain HOTS	Upgrade Categories
M1	4.8	0.71	Height
M2	4.5	0.64	Medium
M3	4.7	0.70	Height
M4	4.6	0.62	Medium
M5	4.9	0.73	Height
M6	4.4	0.65	Medium
M7	4.5	0.63	Medium
M8	4.6	0.61	Medium

Table 5 shows a positive correlation between the quality of the project product and the increase in HOTS (Pearson r = 0.82, p<0.05). Students who are more active and creative in projects tend to have higher N-gains. M5 (with the highest product score of 4.9) explained in an interview: *"I am very involved in experimental design and data analysis. I feel like every decision we make forces me to think critically. A good product is a reflection of a mature thought process."*

In addition, students were also asked to reflect on the three most important HOTS they mastered after the project. The results of the FGD showed that 75% of students (6 out of 8) cited the ability to evaluate evidence as the main achievement, followed by designing experiments (63%) and making data-driven decisions (50%). No student mentioned memorizing concepts as the main achievement. This confirms that project-based learning effectively shifts the focus from memorization to high-level application.

The quality of the project's products (average 4.6 out of 5) and its positive correlation with the increase in HOTS (r=0.82) suggest that the product is not just a final product, but a reflection of a high-level thought process during the project. These findings support the theory (Anggawa & Wartini, 2026) that "personally meaningful projects increase cognitive engagement and perseverance." Students are able to produce biofertilizers with high viability (10^8 CFU/ml) and conduct production cost analysis, which is a form of HOTS integrated with real context. This is in line with the opinion (Riswanto, 2025) that HOTS should be measured through authentic tasks, not just written tests. In addition, students' ability to present and defend products in front of reviewers reflects HOTS's mastery of the level of evaluation and scientific communication. Thus, good product quality not only indicates technical success, but also pedagogical success in developing the high-level thinking skills of UISU Biology Education students.

CONCLUSION

Based on the results of the study, it was concluded that project-based biotechnology learning was effective in improving the Higher Order Thinking Skills (HOTS) of eight Biology Education students at the Islamic University of North Sumatra. The increase was shown by an N-gain of 0.66 (medium category) and a very large effect size (Cohen's d=2.86). The HOTS development process takes place dynamically through five stages: dealing with ambiguity, experimental failure, argumentative collaboration, creative freedom, and metacognitive reflection. The quality of the project product (biofertilizer) was positively correlated with the increase in HOTS (r=0.82). Project-based learning has been proven to be able to sharpen students' analysis, evaluation, and creative skills significantly. Therefore, this model is recommended to be widely implemented in Biotechnology courses in the Biology Education environment of UISU and other universities.

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