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Preparation of Student Activity Sheets Results of Research on the Effect of Crude *Bacillus subtilis* Enzyme Concentration on Sugar and Ethanol Content from Sugarcane Bagasse Fermentation Using *Zymomonas mobilis* as Biology Teaching Materials

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ABSTRACT

Student Activity Sheets (SAS) are teaching materials that are systematically and interestingly arranged in the learning process. In this case, the results of the study are used as material for compiling SAS. The study aims to determine the potential of SAS teaching materials from the results of the study on the effect of crude *Bacillus subtilis* enzyme concentration on sugar and ethanol content from bagasse fermentation using *Zymomonas mobilis*, the effect of crude *Bacillus subtilis* enzyme concentration on sugar and ethanol content from bagasse fermentation using *Zymomonas mobilis*, and the concentration of crude *Bacillus subtilis* enzyme that has the most influence on sugar and ethanol content from bagasse fermentation using *Zymomonas mobilis*.

The research was conducted in 2 stages, the first was an experimental study conducted using a Completely Randomized Design (CRD) with the independent variable being the concentration of crude *Bacillus subtilis* enzyme, namely 0; 2.5; 5; 7.5; 10; 12.5; 15; 17.5% and the dependent variable being the sugar and ethanol content of the fermented sugarcane bagasse using *Zymomonas mobilis* . Measurement of sugar content used the DNS method, while measurement of ethanol content used an alcohol meter. Second, the preparation of SAS used the ADDIE development model which was modified to ADDE. The data from the experimental research results were analyzed using the One Way ANOVA test, while the preparation of SAS was analyzed using quantitative descriptions.

The results of the study showed that the SAS results of the study on the sugar and ethanol content of the results of the study of sugarcane bagasse using *Zymomonas mobilis* were categorized as very good to be used as teaching materials for biotechnology materials. The concentration of crude *Bacillus subtilis* enzyme that had the most influence on the sugar and ethanol content of the fermentation results of

sugarcane bagasse using *Zymomonas mobilis* was in treatment 4 with a concentration of 10%, namely 0.67 g/mL and the highest ethanol content, namely 1.89%.

Introduction

Student Activity Sheets (SAS) are activity sheets for the learning process to find science concepts either through theory, demonstration, or investigation accompanied by clear instructions and work procedures to train thinking skills and science process skills in completing tasks according to the learning indicators to be achieved (Firdaus & Wilujeng, 2018). The development of SAS is a fundamental and important aspect to create meaningful learning. One of the SAS developments is in the form of SAS based on research results. Research related to the manufacture of bioethanol from various substrates has been widely carried out, but not many have utilized the results of this research as teaching materials. Teaching materials are all materials, both written and unwritten, that are used to help students learn, especially during the learning process (Puryadi, et al, 2018; Magdalena et al., 2020). References in making teaching materials are not only based on books but can also be based on the results of research that has been carried out that relate to the competencies and goals to be achieved by students. The preparation of research results must still be adjusted to the cognitive development of participants. In the development of teaching materials, there are various aspects that can be used as benchmarks, namely: 1) A concept is an idea or concept. 2) A principle is a basic truth as a starting point for thinking or is a guide to doing or implementing something. 3) A fact is something that has happened or that has been done or experienced. 4) A process is a series of changes, development movements. 5) Value is a pattern, measurement or is a type or model (Magdalena et al., 2020).

In general, teaching materials can be divided into printed and non-printed teaching materials (Afnidar et al., 2019), can also be grouped into four, namely (1) Printed materials including handouts, books, modules, Student Worksheets, pictures. (2) Hearing teaching materials (audio) such as cassettes, radio, and audio CDs. (3) Hearing-visual teaching materials (audio visual) such as video CDs, films. and (4) Interactive teaching materials such as interactive CDs (Arsanti, 2018). Teaching materials affect the success of students in the learning process in addition to the role of a teacher, so it is necessary to formulate teaching materials that are able to support the implementation of good education, one of which is SAS based on research results. One of the materials in high school whose learning can be supported by SAS teaching materials based on research results is biotechnology material related to the use of microbes in fermentation. Microbes that are often used in food fermentation and bioethanol production are Saccharomyces cerevisiae and *Zymomonas mobilis*. Both microbes each have advantages in carrying out fermentation.

Zymomonas mobilis is a facultative anaerobic bacteria that can utilize sucrose, glucose, and fructose as energy sources, and has a high growth rate and resistance to ethanol concentrations of around 16%. This bacteria can convert sugar into ethanol and CO2 through glycolytic "Entner-Duodoroff", where oxygen is not involved in the fermentation process to avoid inhibition of respiration. This bacteria is also a type of bacteria that is tolerant to ethanol, so it has a higher glucose absorption rate and is able to produce ethanol under anaerobic conditions (Kusmiyati et al., 2016). Meanwhile, Saccharomyces cerevisiae can break down the glucose content well and can last longer in the fermentation process. (Cahyaningtiyas & Sindhuwati, 2023).

In biotechnology material in high school, there are many materials that can be used in fermentation, including palm sap, fruits, agricultural waste, and industrial waste, including waste/bagasse in sugar making. Bagasse contains lignocellulose, 40% cellulose, 29% hemicellulose, 13% lignin, and silica

(Zuniar & Purnomo, 2016). The content of lignocellulose, 40% cellulose, and 29% hemicellulose has the potential to be developed into bioethanol, with the help of enzymes that can convert cellulose into glucose, including the cellulase enzyme from Bacillus subtilis. These bacteria are able to produce the cellulase enzyme when placed on materials containing cellulose (Danilova & Sharipova, 2020). Based on the importance of teaching materials on biotechnology material in high school based on research results, this study aims to determine the potential of SAS teaching materials from the results of research on the effect of crude *Bacillus subtilis* enzyme concentration on sugar and ethanol content from fermented sugarcane bagasse using *Zymomonas mobilis* .

Method

Stage

This research consists of 2 stages, namely experimental research using RAL with 1 factor, namely the concentration of cellulase enzyme consisting of treatments of 2.5%; 5%; 7.5%; 10%; 12.5%; 15%; and 17.5%. and the preparation of SAS using the ADDIE model. Data analysis in experimental research using ANOVA, while the analysis of SAS preparation using descriptive analysis.
Steps taken:

a. Experimental research
   1. Cultivation of *Bacillus subtilis* and *Zymomonas mobilis* Bacteria

   Nutriant Agar (NA) media is made by weighing 2 grams of NA media into a beaker containing 100 mL of distilled water. The solution is heated while stirring so that the NA powder dissolves completely. After that, the media is put into a test tube and a petri dish. In the test tube, the mouth of the test tube is covered with cotton that has been wrapped in sterile gauze. The media is sterilized by autoclaving at a temperature of 121℃ for 15 minutes, then made to tilt and left for 24 hours in the incubator. After solidifying, it can be used to grow bacteria using an ose wire (Sholihati et al., 2015).

   2. Preparation of Sugarcane Bagasse Powder

In this process, the sugarcane bagasse is dried in the sun, after drying, the sugarcane bagasse is blended and then soaked in 1 M NaOH solution for 16 hours, the sample is then washed using distilled water until a neutral pH (7) is obtained. After the pH is neutral, the sugarcane bagasse powder is dried using an oven at a temperature of 105°C to remove water content (Rahmadani et al., 2017)

   3. Making Crude Extract of Cellulase Enzyme

Crude cellulase enzyme is made by solid substrate fermentation. A total of 10 grams of pretreated bagasse powder and 50 mL of nutrient solution are put into a 500 mL Erlenmeyer flask and sterilized by autoclave. Next, the Bacillus subtilis suspension is taken using an ose wire into a medium containing sterilized bagasse powder, then incubated for 3 days. After the incubation process, the crude cellulase enzyme that has been produced is extracted using 150 mL of 0.01% tween 80 solution for enzyme harvesting, then a diamagnetic stirrer for 30 minutes (Retnoningtyas et al., 2013). Furthermore, the sediment and fermentation liquid are separated using centrifugation at a speed of 3000 rpm for 10 minutes. The supernatant from the centrifugation is obtained and will be used in the enzymatic hydrolysis stage. (Kartikasari et al., 2013)

   4. Hydrolysis Process

250 g of sugarcane bagasse powder was dissolved in 2.5 L of distilled water and then cooked until boiling. The extract was then taken. 100 mL of sugarcane bagasse extract was put into a 100 mL bottle and sterilized using an autoclave for 15 minutes at a temperature of 121℃. After cooling,

crude Bacillus subtilis enzyme was added according to the concentration treatment, namely (0%), (2.5%), (5%), (7.5%), (10%), (12.5%), (15%), and (17.5%) aseptically and made three times (24 bottles of 100 mL), then stirred using a sterile glass stirrer. The 100 mL bottle was closed using sterile cotton, coated with aluminum foil and tied with a rubber band then incubated for 24 hours in a room with a temperature of 30°C. The hydrolysis results were measured using the DNS (Dinitrosalicylic acid) method to determine the sugar content (Widyaningrum & Parahadi, 2020).

5. Bioethanol Production

Hydrolyzed bagasse (24 bottles of 100 mL treated with Bacillus subtilis crude enzyme), each added with 10% Zymomonas mobilis bacteria, then each fermented for 4 days for bioethanol production (Kodri et al., 2013). The results of the fermentation treatment were measured for reducing sugar content using the DNS method to compare sugar content before and after treatment with Zymomonas mobilis . The results of fermentation with Zymomonas mobilis were distilled, the distillate results were measured for ethanol content using an alcohol meter (Widyaningrum & Parahadi, 2020).

b. Steps for Compiling SAS Based on Research Results

The research procedures carried out in the development using the ADDE model are analysis, design, development, and evaluation (Latifah & Widjajanti, 2017).

1. Analysis Stage

a) Analysis of Teaching Material Potential

This analysis aims to test the potential of teaching materials as a basis for compiling SAS.

b) Content Analysis

This analysis is carried out to test the content based on the curriculum that refers to core competencies (KI) and basic competencies (KD).

c) Needs Analysis

Needs analysis aims to collect data/information regarding problems experienced by students during the learning process in terms of material relevance, learning strategies, and learning conditions.

d) Objective Analysis

Objective analysis contains an assessment of core competencies, basic competencies, learning indicators, and learning objectives.

2. Design Stage

a) SAS Design

This stage is carried out by creating a SAS framework in the form of a title, basic competencies, learning indicators, learning objectives, materials, supporting information, and tasks or work steps.

This design draft is in the form of a description of the SAS that will be developed according to the specified topic.

3. Development Stage

1) SAS Creation
   Collecting data, references, and literature studies regarding the material to be developed in the SAS.
2) Drafting
   Compiling the SAS according to the framework that has been made. Then, the results are consulted with the supervising lecturer.
3) Revision
   Improvements are made according to suggestions from material experts, media experts, and supervising lecturers.
4) Editing
   This stage is the collection of the final assessment results from experts on the SAS that has been compiled to

then be revised again to make it better.

4. Evaluation Stage (Evaluation)
   This stage is the final assessment stage of the SAS carried out by the validator.

Data Analysis

1. Experimental Data Analysis

The data obtained are sugar and ethanol levels based on the effect of the concentration of crude *Bacillus subtilis* enzyme from fermented sugarcane bagasse using *Zymomonas mobilis* . Data analysis using SPSS assistance with regression tests to determine the effect of the concentration of crude *Bacillus subtilis* enzyme from fermented sugarcane bagasse using *Zymomonas mobilis*, then a variance analysis (ANOVA) test was carried out to see the differences in each treatment.

2. Analysis of Potential Teaching Material Results

The data from the analysis of the potential of research results as teaching materials are qualitative data. This qualitative data is then analyzed descriptively. Descriptive research is a research method that attempts to describe and interpret objects according to what they are. This qualitative data is arranged according to the steps of analyzing the potential of research results as teaching materials.

The data obtained from the results of the instrument assessment carried out by the validator, then the total score is calculated and the results can be seen from the intervals provided so that they can be calculated in quantitative form. The calculation of the score from the respondents' answers is strongly agree (SS) = 4, agree (S) = 3, disagree (TS) = 2, strongly disagree (STS) = 1. The calculation is done with the index (%) = total score/maximum score × 100. The assessment interval used to determine the level of validity of the product made is as follows.:

Table 1. Index and Assessment Interval

No	Indeks	Rating
1	0% - 20%	Very bad
2	21% - 40%	Not good
3	41% - 60%	Not good
4	61% - 80%	good
5	81% - 100%	Very good

(Widagdo et al., 2020)

Results and Discussion

1. Experimental Research Results

a. Sugarcane bagasse reducing sugar content before and after pre-treatment

The sugarcane bagasse substrate reducing sugar content before and after pre-treatment was measured in this study as follows:

Table 2. Sugarcane bagasse reducing sugar content before and after pretreatment

Replication	Reducing Sugar Level (g/mL)	
	before Pre-treatment	After Pre-treatment
1	0.26	0.30
2	0.27	0.36
3	0.35	0.31
Average	0.29	0.32

b. Sugarcane bagasse reducing sugar content after adding crude *Bacillus subtilis* enzyme

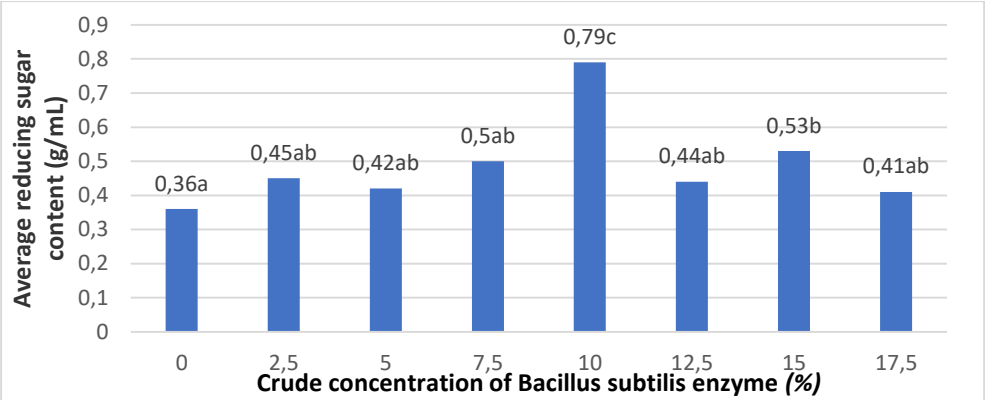

Figure 1. Diagram of the effect of crude *Bacillus subtilis* enzyme concentration on the reducing sugar content of sugarcane bagasse.

c. Sugar reduction content of sugarcane bagasse after fermentation using Zymomonas mobilis

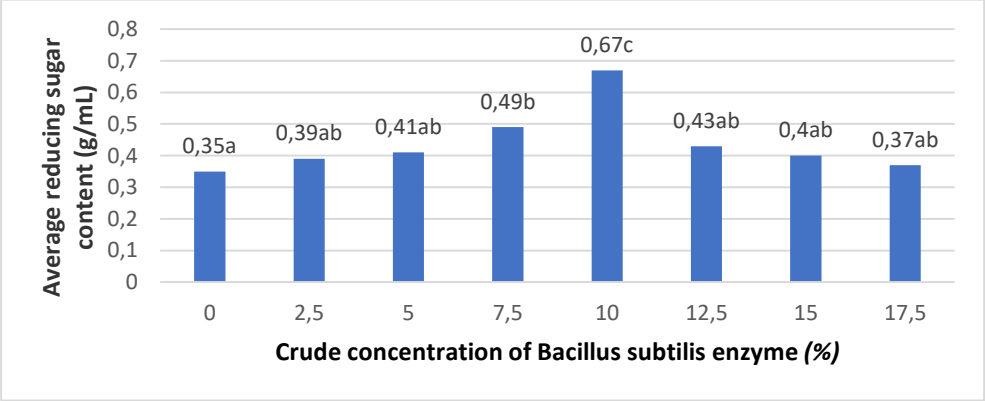

Figure 2. Diagram of the effect of crude *Bacillus subtilis* enzyme concentration on the reducing sugar content of sugarcane bagasse after *Zymomonas mobilis* fermentation.

d. Bioethanol Content Test Results

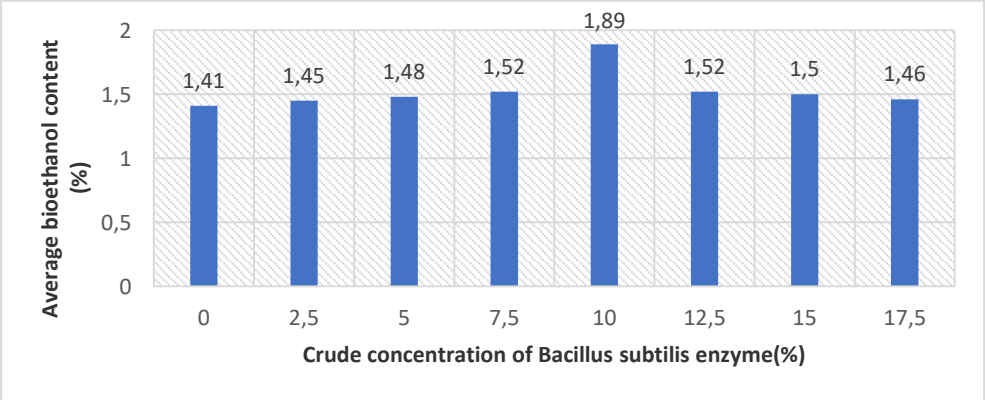

Figure 3. Diagram of the effect of crude *Bacillus subtilis* enzyme concentration on ethanol content

Based on the ANOVA test, the significance value was > 0.05 (0.54), which means that the bioethanol content of various crude enzyme concentrations is not significantly different.

2. Educational Outcomes

Student Activity Sheets on the results of research on the effect of crude *Bacillus subtilis* enzyme concentration on sugar and ethanol content from fermented sugarcane bagasse using *Zymomonas mobilis* as biology teaching materials for grade XII students on biotechnology material are compiled using 4 stages, namely the analysis stage (analyze), design (design), development (development), and evaluation (evaluation).

a. Analysis Stage (Analyze)

1. Analysis of Potential Teaching Materials

Analysis of potential teaching materials is carried out by preparing materials for compiling student activity sheets related to biotechnology material

that can be studied as teaching materials, namely the effect of crude *Bacillus subtilis* enzyme concentration on sugar and ethanol levels from fermented sugarcane bagasse using *Zymomonas mobilis* which was carried out at the Biology Laboratory of Ahmad Dahlan University. The results of this study can be identified as teaching materials that will later be used for classroom learning if they meet the requirements and criteria in order to create effective learning. According to (Yuniarti et al., 2018) must meet several criteria as follows:

Table 3. Requirements for research results as learning resources for high school biology

No	Aspect	Description
1.	Clarity on potential availability of objects and issues raised	The potential of this research object is relevant to the achievement of competency standards and basic competencies. The problems raised are fermentation using sugarcane waste as a raw material for bioethanol with the help of microorganisms and the need for variations in interesting teaching materials in biotechnology materials for grade XII high school students..
2.	Compliance with learning objectives	The results of the study are in accordance with the objectives of biology learning in KD 3.10, namely: a. Students are able to explain the meaning of biotechnology. b. Students are able to distinguish between conventional and modern biotechnology. c. Students are able to analyze the application of biotechnology in everyday life.
3.	Suitability of materials	The suitability of research with biotechnology material regarding its application in daily life, namely the fermentation of sugarcane bagasse which produces bioethanol.
4.	Clarity of the information disclosed	Clarity of information raised from the research results in the form of: a. Facts that bagasse contains cellulose. b. Principles that bagasse waste can be used as raw materials for making bioethanol. c. Procedures work methods that are in accordance with scientific methods related to the use of bagasse waste as a raw material for making bioethanol.
5.	Clarity of exploration guidelines	Clarity of activity guidelines related to research procedures. This research was conducted experimentally,

No	Aspect	Description
		namely by collecting data, analyzing data and analyzing research results..
6.	Clarity of expected gains	The report of the results of this study can help students in achieving learning objectives from the following aspects: a. Cognitive Students can learn biotechnology material about making bioethanol using bagasse waste as raw material. b. Affective Increase students' curiosity regarding the process of making bioethanol from bagasse waste and students have a sense of responsibility to complete the tasks in the SAS. c. Psychomotor Students are able to communicate the results of discussions related to the process of making bioethanol from bagasse and related to the tasks in the SAS.

2. Identification of Potential Research Processes and Products as Biology Teaching Materials for Grade XII Students

Identification of research processes as teaching materials is based on work procedures that are in accordance with the steps of the scientific method.

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Table 4. Results of identification of research processes as learning resources

No	Process	Description
1.	Formulation of the problem	1. Are the results of the study on the effect of crude *Bacillus subtilis* enzyme concentration on sugar and ethanol content from bagasse fermentation using *Zymomonas mobilis* potentially used as biology teaching materials for biotechnology materials? 2. Does the concentration of crude *Bacillus subtilis* enzyme affect the sugar and ethanol content in bagasse fermentation using *Zymomonas mobilis* ? 3. What is the concentration of crude *Bacillus subtilis* enzyme that has the most effect on sugar and ethanol content in bagasse fermentation using *Zymomonas mobilis* ?
2.	Formulation of research objectives	1. To determine the potential results of the study of the effect of crude *Bacillus subtilis* enzyme concentration on sugar and ethanol levels in bagasse fermentation using *Zymomonas mobilis* as a biology teaching material for biotechnology materials. 2. To determine the effect of crude *Bacillus subtilis* enzyme on sugar and ethanol levels in bagasse fermentation using *Zymomonas mobilis* . 3. To determine the concentration of crude *Bacillus subtilis* enzyme that has the most effect on sugar and ethanol levels in bagasse fermentation using *Zymomonas mobilis* .
3.	Formulation of hypothesis	1. Student Activity Sheet from the results of the study on the effect of crude *Bacillus subtilis* enzyme concentration on sugar and ethanol levels from bagasse fermentation using *Zymomonas mobilis* can be used as biology teaching materials for biotechnology materials.

No	Process	Description
		2. The concentration of *Bacillus subtilis* has the potential to influence sugar and ethanol levels in bagasse fermentation using *Zymomonas mobilis* maximally. 3. The concentration of crude *Bacillus subtilis* enzyme that has the most influence on sugar and ethanol levels in bagasse fermentation using *Zymomonas mobilis* is at a concentration of 10%.
4.	Preparation of research procedures	1. Preparation of tools and materials 2. Cultivation of *Bacillus subtilis* and *Zymomonas mobilis* bacteria 3. Preparation of sugarcane bagasse powder 4. Preparation of nutrient solution 5. Preparation of tween solution 6. Preparation of crude extract of cellulase enzyme 7. Preparation of starter 8. Hydrolysis process 9. Preparation of DNS solution 10. Measurement of reducing sugar content 11. Bioethanol production 12. Distillation process 13. Measurement of bioethanol content
5.	Implementation of activities	The research was conducted at the UAD Biology Laboratory and the preparation of student activity sheets was carried out at home. The research was conducted in February 2022 - April 2022.
6.	Data collection and analysis	Data collection was carried out by measuring the reducing sugar content using a spectrophotometer, measuring the bioethanol content with a distillation process which was then measured with an alcohol meter, then to determine the effect of each treatment, data analysis was carried out using SPSS with the One Way Anova test to determine the effect of each treatment..
7.	Discussion of research results	Discussion of research results in the form of facts and thoughts from research results supported by relevant theories. This study discusses the results of research on the effect of crude *Bacillus subtilis* enzyme concentration on sugar and ethanol content of sugarcane bagasse.
8.	Drawing conclusions	1. Student Activity Sheet from the results of the study on the effect of crude *Bacillus subtilis* enzyme concentration on sugar and ethanol levels from bagasse fermentation using *Zymomonas mobilis* can be used as biology teaching materials for biotechnology materials. 2. The concentration of *Bacillus subtilis* has the potential to influence sugar and ethanol levels in bagasse fermentation using *Zymomonas mobilis* maximally. 3. The concentration of crude *Bacillus subtilis* enzyme that has the most influence on sugar and ethanol levels in bagasse fermentation using *Zymomonas*

No	Process	Description
		mobilis is at a concentration of 10%.

3. Identification of Research Products as Biology Teaching Materials

The research results are in the form of research facts, then generalized into concepts and principles as in the following table 5.

Table 5. Results of identification of research products as biology teaching materials

No	Fact	Concept	Principle
1.	Sugarcane pulp contains carbohydrates	Sugarcane waste contains carbohydrate compounds	Sugarcane bagasse waste can be used as raw material for making bioethanol
2.	The crude concentration of *Bacillus subtilis* enzyme that produces the highest reducing sugar content is in the P4 treatment with a concentration of 10%.	Crude enzyme *Bacillus subtilis* can hydrolyze cellulose	Crude *Bacillus subtilis* enzyme can increase reducing sugar levels

4. Selection and Modification of Research Results as Teaching Materials

Before the research report is used as teaching materials. The research process in the form of research work procedures needs to be selected and modified first so that it can be adjusted in learning activities.

Table 6. Selection and modification of research results as biology teaching materials

Research Work Procedures	Results of Selection and Modification of Research Work Procedures
1. Preparation of tools and materials 2. Preparation and manufacture of bagasse substrate 3. Measurement of solution pH 4. Pretreatment of bagasse powder using NaOH 5. Hydrolysis process 6. Fermentation process 7. Measurement of bioethanol content	Observing and analyzing several research work procedures as follows: 1. Making sugarcane bagasse substrate 2. Hydrolysis process using crude enzyme *Bacillus subtilis* 3. Fermentation process using *Zymomonas mobilis* 4. Measuring ethanol levels

5. Application and Development of Research Results as Biology Teaching Materials

Application of research results on the effect of crude *Bacillus subtilis* enzyme concentration on sugar and ethanol content from fermentation of sugarcane bagasse waste using *Zymomonas mobilis* as biology teaching materials realized in the form of SAS.

a) Content Analysis

This content is related to the basic competencies used in learning in accordance with the 2013 curriculum.

Table 7. Core competencies and basic competencies

Core Competencies (CC)	Basic competencies (BC)
3.Understand, apply, analyze and evaluate factual, conceptual, procedural and metacognitive knowledge based on curiosity about science, technology, arts, culture and humanities with insights into humanity, nationality, statehood and civilization related to the causes of phenomena and events, and apply procedural knowledge to specific fields of study according to talents and interests to solve problems.	3.10 Analyze the principles of biotechnology and its application as an effort to improve human welfare.

The 2013 Curriculum requires students to be active during learning. Each subject has CC and BC that must be achieved. As for CC and BC 3.10 in biotechnology material up to the C4 (analysis) capability level so that the competency achievement indicator must reach the stage of analyzing biotechnology principles and their application as an effort to improve human welfare.

b) Needs Analysis

This study refers to grade XII students of SMAN 11 Tangerang Regency with biotechnology material as the target in the use of teaching materials, because in the learning process the teaching materials used are textbooks. Information related to the application of biotechnology is still limited, such as making tempeh, tape, or yogurt and students are required to be active in terms of cognitive, affective and psychomotor. So there needs to be teaching materials developed from research results to increase student enthusiasm in carrying out interesting learning activities and making learning more effective and efficient. The teaching materials are in the form of SAS on making bioethanol from sugarcane pulp.

c) Objective Analysis

The objective analysis is carried out by describing the Basic Competencies into learning objectives and indicators that must be achieved by students in the Biotechnology material which is specifically focused on the results of bioethanol research from sugarcane pulp. The following is a description of the Basic Competencies into learning objectives and indicators:

Table 8. Description of basic competencies with indicators and objectives

Basic competencies	Indicator	Learning objectives
3. 10 Analyze the principles of biotechnology and its application as an effort to improve human welfare..	1. Explain the meaning of biotechnology. 2. Differentiate between conventional and modern biotechnology. 3. Analyze the application of biotechnology in everyday life.	1. Students are able to explain the meaning of biotechnology. 2. Students are able to differentiate between conventional and modern biotechnology. 3. Students are able to analyze the application of biotechnology in everyday life.

b. Design Stage

1. SAS Design

The design stage is carried out by creating a SAS framework in the form of:

a) Cover page
Contains the SAS title, agency logo, supporting images and the name of the compiler.
b) Competence
Contains the basic competencies to be achieved by students.
c) Competence Achievement Indicator
Contains a description of indicators based on basic competencies.
d) Learning Objectives
Contains the learning objectives to be achieved by students
e) Material
Contains general biotechnology material along with its types, how the fermentation process works and bacteria used in the experiment.
f) Supporting Information
Contains images to clarify understanding of the material.
g) Work Steps and Tasks
Contains the work steps that must be carried out by students and there are several tasks or questions that must be answered by students.
h) Bibliography
Contains references from journals used in compiling SAS.

2. Design Document Design

SAS for making bioethanol from sugarcane waste is arranged in the form of an activity sheet which is then printed. The arrangement of this SAS uses the Canva application which is designed as creatively as possible using attractive font types and sizes and a good combination of colors so that it can attract students to use it in the learning process.

c. Development Stage

1) Making SAS
This stage is carried out by collecting research data, references, and literature studies related to the material to be developed in the SAS, especially biotechnology.

2) Making a Draft
At the drafting stage, the preparation is carried out according to the previously made design. Then the draft SAS draft is submitted to the supervising lecturer in order to get suggestions for improving the SAS. Some suggestions given by the supervising lecturer include the following:

Table 9. Input and suggestions from the supervising lecturer and follow-up actions taken

No	Input/Suggestions	Follow-up
1.	Correction of sentence writing in SAS	The incorrect sentence has been corrected
2.	SAS is made in accordance with the components of a good SAS	SAS has been revised according to components
3.	Indicators are placed before learning objectives	Indicators have been placed above the learning objectives

3) SAS Validation
At the SAS validation stage, the product is consulted with two expert material and media validator lecturers to test the instruments in the SAS assessment related to the aspects to be assessed.

4) Editing
The SAS editing stage is carried out based on the assessment of the validator lecturer to improve the quality of the product developed as a biology teaching material for grade XII of high school. There are two validator lecturers, namely the material expert validator and the media expert validator to assess the SAS components including the appropriateness of the content, language, presentation, and graphics. The results of the assessment from the material expert validator lecturer can be seen in Table 22 and the results of the assessment from the

media expert validator lecturer can be seen in Table 10.

Table 10. Results of validator 1 assessment

No	Component Aspects	Percentage (%)	Category
1.	Content Suitability	92	Very Good
2.	Language	87,5	Very Good
3.	Presentation	100	Very Good
4.	Graphics	75	Good
	Average	88,63	Very Good

The component assessment on the aspect of the feasibility of the SAS content shows a percentage of 92% so it is categorized as very good. On the linguistic aspect, the SAS shows a percentage of 87.5% so it is categorized as very good. On the presentation aspect, the SAS shows a percentage of 100% so it is categorized as very good. On the graphic aspect, the SAS shows a percentage of 75% so it is categorized as good.

Based on the results of the assessment of validator 1, it shows the overall percentage of component aspects of 88.63% and is included in the very good category. So it can be seen that the results of the validator's assessment of the SAS product that has been prepared are included in the very good category and the validator stated that the SAS is suitable for use as a biology teaching material for senior high school class XII on biotechnology material. The suggestion given by validator lecturer 1 is to replace the paper used in the SAS.

Table 11. Results of validator 2 assessment

No	Component Aspects	Percentage (%)	Category
1.	Content Suitability	83	Very Good
2.	Language	87,5	Very Good
3.	Presentation	75	Good
4.	Graphics	66	Good
	Average	77,88	Good

The component assessment on the aspect of the feasibility of the SAS content shows a percentage of 83% so it is categorized as very good. In the linguistic aspect, the SAS shows a percentage of 87.5% so it is categorized as very good. In the presentation aspect, the SAS shows a percentage of 75% so it is categorized as good. In the graphic aspect, the SAS shows a percentage of 66% so it is categorized as good. Based on the results of the assessment of validator 2, the overall percentage of the component aspect is 77.88% and is included in the good category. So it can be seen that the results of the validator's assessment of the SAS product that has been prepared are included in the good category and the validator stated that the SAS is suitable for use as a biology teaching material for senior high school class XII on biotechnology material. The suggestions given by validator lecturer 2 are that the product is equipped with a table of contents, pictures/photos/illustrations are added so that they support the concept presented, improvements to the writing of inappropriate words, improvements to learning indicators with existing questions, covers are made by illustrating the processing of sugarcane pulp into bioethanol.

5) Revision

Revisions were made according to input or suggestions from validator lecturer 1 and validator lecturer 2. The following are some of the results of improvements based on input or suggestions from material expert lecturers and media experts which are included in the attachment.

d. Improvement Stage (Evaluation)

At the improvement stage, it is done to improve the teaching materials in the form of SAS. Improvements are made according to input/suggestions from the supervising lecturer, material expert validator and media expert validator. The following are the results of the improvements made which can be seen in the attachment.

Discussion

Based on the results of measuring the reducing sugar content after the fermentation process using *Zymomonas mobilis* , the average highest reducing sugar

content was treatment 4 (10% concentration) of 0.67 g/mL, while the average lowest reducing sugar content was 0.35 g/mL in treatment K (0% concentration). The average reducing sugar content obtained after *Zymomonas mobilis* fermentation decreased when compared to after crude cellulose enzyme hydrolysis. This decrease in sugar content is because the sugar content has been utilized by *Zymomonas mobilis* to be converted into ethanol and for cell growth (Retnoningtyas et al., 2013).

The results of measuring the bioethanol content using an alcohol meter showed that the highest bioethanol content was in treatment 4 (10% concentration) of 1.89% and the lowest average bioethanol content was in the Control (0% concentration) of 1.41%. The more reducing sugar that can be utilized by *Zymomonas mobilis* , the higher the ethanol content produced. However, there was a decrease in ethanol content in treatment 5 (12.5% concentration), treatment 6 (15% concentration) and treatment 7 (17.5% concentration). This happened because the nutrients were decreasing, and the higher ethanol content could inhibit bacterial growth (Kartikasari et al., 2013).

Discussion of the preparation of SAS

Based on the assessment conducted by the expert material validator lecturer and media expert related to the aspects of the SAS components which include the feasibility of content, language, presentation and graphics. There are assessment results obtained from validator lecturer 1 (material expert) on the feasibility aspect of the SAS content getting a percentage of 92% so that it is categorized as very good. This shows that the feasibility aspect of the content including the SAS material is in accordance with the basic competencies and objectives to be achieved by students, and the material presented is accurate based on research facts and uses relevant references. In the linguistic aspect, the SAS gets a percentage of 87.5% so that it is categorized as very good. This shows that the SAS has clear information in accordance with the rules of the General Spelling Guidelines for Indonesian (PEUBI) and uses effective and efficient sentences so that it is easy to read and understand by students. In the presentation aspect, the SAS gets a percentage of 100% so that it is categorized as very good. This shows that the SAS has a complete structure starting from the title, identity, basic competencies, indicators, objectives, main material, supporting information, work steps or tasks to be completed by students. In the graphic aspect, the SAS received a percentage of 75% so that it was categorized as good. This shows that the preparation of the SAS has used good and attractive font types and sizes, has an attractive image layout and is related to the material and has an attractive display design.

Based on the research results obtained from validator lecturer 2 (media expert) on the aspect of the feasibility of the SAS content, the percentage was 83% so it was categorized as good. This shows that the aspect of the feasibility of the content which includes the SAS material is in accordance with the basic competencies and objectives to be achieved by students and the material presented is accurate based on facts and research results and uses relevant references. In terms of language, the SAS has clear information in accordance with the rules of the General Spelling Guidelines for the Indonesian Language (PEUBI) and uses effective and efficient sentences so that it is easy to read and understand by students. In terms of presentation, the SAS gets a percentage of 75% so it is categorized as good. This is because the product is not equipped with a table of contents and there are questions that do not match the learning indicators. In terms of graphics, the SAS gets a percentage of 66% so it is categorized as good. This is because there are no pictures/photos/illustrations presented and the cover section does not illustrate the processing of sugarcane pulp into bioethanol..

Based on the assessment results carried out by validator lecturers 1 and 2, the average is 83.25%, meaning that the entire contents of the SAS are categorized as very good. This is in accordance with the theory of Pradana & Mawardi (2021) that the results of the validation test are said to

be suitable for testing if the minimum percentage is ≥ 61%.

Conclusion

Student Activity Sheets (SAS) results of the study on the effect of crude *Bacillus subtilis* enzyme concentration on sugar and ethanol content from bagasse fermentation using *Zymomonas mobilis* have the potential as biology teaching materials for grade XII students on biotechnology. The concentration of crude *Bacillus subtilis* enzyme affects the sugar and ethanol content produced by bagasse with the help of *Zymomonas mobilis*. The concentration of crude *Bacillus subtilis* enzyme that has the most effect on sugar and ethanol content in bagasse fermentation using *Zymomonas mobilis* is a concentration of 10% (treatment 4).

References

Afnidar, T., Hartinawati, H., & Jamaludin, J. (2019). Developing of Learning Material to Create New Processes and Products Learning Material Conventional, Blended Learning and Fully Online at Distance Learning, Open University, Jakarta Indonesia. International Journal for Digital Society, 10(1), 1442–1449. https://doi.org/10.20533/ijds.2040.2570.2019.0179

Arsanti, M. (2018). Pengembangan Bahan Ajar Mata Kuliah Penulisan Kreatif Bermuatan Nilai-Nilai Pendidikan Karakter Religius Bagi Mahasiswa Prodi Pbsi, Fkip, Unissula. KREDO : Jurnal Ilmiah Bahasa Dan Sastra, 1(2), 71–90. https://doi.org/10.24176/kredo.v1i2.2107

Cahyaningtiyas, A., & Sindhuwati, C. (2023). Pengaruh Penambahan Konsentrasi Saccharomyces Cerevisiae Pada Pembuatan Etanol Dari Air Tebu Dengan Proses Fermentasi. Distilat: Jurnal Teknologi Separasi, 7(2), 89–94. https://doi.org/10.33795/distilat.v7i2.207

Danilova, I., & Sharipova, M. (2020). The practical potential of bacilli and their enzymes for industrial production. Frontiers in Microbiology, 11(August). https://doi.org/10.3389/fmicb.2020.01782

Firdaus, M., & Wilujeng, I. (2018). Pengembangan LKPD inkuiri terbimbing untuk meningkatkan keterampilan berpikir kritis dan hasil belajar peserta didik. Jurnal Inovasi Pendidikan IPA, 4(1), 26–40. https://doi.org/10.21831/jipi.v4i1.5574

Kartikasari, D. S., S. Nurhatika., dan A. M. (2013). Potensi Alang-alang (Imperata cylindrica (L.) Beauv) dalam Produksi Etanol Menggunakan Bakteri *Zymomonas mobilis*. Jurnal Sains Dan Seni Pomits, 2(2), 127–131.

Kartikasari, S. D., Nurhatika, S., & Muhibuddin, A. (2013). Potensi Alang-Alang (Imperata cylindrica (L.) Beauv.) Dalam Produksi Etanol Menggunakan Bakteri *Zymomonas mobilis*. Jurnal Sains Dan Seni Pomits, 2(2), 1–11.

Kusmiyati, Hadiyanto, H., & Kusumadewi, I. (2016). Bioethanol production from iles-iles (Amorphopallus campanulatus) flour by fermentation using *Zymomonas mobilis*. International Journal of Renewable Energy Development, 5(1), 9–14. https://doi.org/10.14710/ijred.5.1.9-14

Latifah, U. H., & Widjajanti, D. B. (2017). Pengembangan bahan ajar statistika dan peluang berbasis multiple intelligences berorientasi pada prestasi, pemecahan masalah, dan rasa ingin tahu. Jurnal Riset Pendidikan Matematika, 4(2), 176. https://doi.org/10.21831/jrpm.v4i2.13083

Magdalena, I., Prabandani, R. O., Rini, E. S., Fitriani, M. A., & Putri, A. A. (2020). Analisis Pengembangan Bahan Ajar. Jurnal Pendidikan Dan Ilmu Sosial, 2(2), 170–187. https://ejournal.stitpn.ac.id/index.php/nusantara

Pradana, F. A. P., & Mawardi, M. (2021). Pengembangan Instrumen Penilaian Sikap Disiplin Menggunakan Skala Likert dalam Pembelajaran Tematik Kelas IV SD. Fondatia, 5(1), 13–29.

https://doi.org/10.36088/fondatia.v5i1.1090

Puryadi*, Satutik Rahayu, S. (2018). Pengaruh Model Pembelajaran Direct Instruction Berbantuan Bahan Ajar Berbasis Kontekstual Terhadap Hasil Belajar IPA Terapan Siswa Kelas X SMKN 4 Mataram Tahun Ajaran 2015/2016. Jurnal Pendidikan Fisika Dan Teknologi, 4(1). https://doi.org/10.29303/jpft.v4i1.329

Rahmadani, S., Muria, S. R., & Utami, S. P. (2017). Produksi Bioetanol Dari Mahkota Nanas Menggunakan Bakteri *Zymomonas mobilis* Dengan Variasi Konsentrasi Inokulum Dan Penambahan Nutrisi. Fteknik, 4(2), 1639–1642.

Retnoningtyas, E. S., Antaresti, & Aylianawati. (2013). Aplikasi Crude Enzim Selulase Dari Tongkol Jagung (Zea Mays L) Pada Produksi Etanol Dengan Metode Simultaneous Saccharification And Fermentation (Ssf) , Reaktor, 14(4), 272–276.

Sholihati, A. M., Baharuddin, M., & Santi, S. (2015). Produksi dan Uji Aktivitas Enzim Selulase dari Bakteri Bacillus subtilis. Al-Kimia, 3(2), 78–90.

Widagdo, B. W., Handayani, M., & Suharto, A. (2020). Dampak pandemi COVID-19 terhadap perilaku peserta didik pada proses pembelajaran daring menggunakan metode pengukuran skala likert (studi kasus di Kabupaten Tangerang Selatan). Jurnal Teknologi Informasi ESIT, 15(2), 63–70.

Widyaningrum, T., & Parahadi, M. (2020). Bioethanol Levels of Dragon Fruit (Hylocereus polyrhizus) Peel with the Addition of Blend Crude Cellulase Enzyme from Trichoderma reesei and Aspergillus niger. Journal of Tropical Biodiversity and Biotechnology, 5(1), 1. https://doi.org/10.22146/jtbb.52189

Yuniarti, R. D., Situmorang, R. P., & Krave, A. S. (2018). Efektivitas Model Joyful Learning dalam Meningkatkan Pemahaman Konsep Siswa SMP dengan Memperhatikan Domain Soal. Jipva, 2, 122. https://doi.org/10.31331/jipva.v2i1.540

Zuniar & Purnomo. (2016). Pengaruh Campuran Ampas Tebu dan Tongkol Jagung sebagai Media Pertumbuhan terhadap Kandungan Nutrisi Jamur Tiram Putih (Pleurotus ostreatus). Jurnal Sains Dan Seni ITS, 5(2), 2–5.