The Asian Journal of Biology Education

ISSN 1447-0209

Volume 13: December 2021

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The Asian Journal of Biology Education

Volume 13: December 2021

Practical Report

A Biochemical Experiment to Assess Cognitive Skills and Procedural Understanding in High School Students

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(Received: 27 October 2020; Accepted for publication: 28 May 2021)

Learning of concepts in biology can be made effective by providing historical perspectives, social context and inclusion of laboratory exercises in regular teaching practice. Among laboratory exercises, biochemical experiments prove to be useful tools to promote biology as a subject of logical reasoning requiring analytical skills. One such investigation of an enzyme-catalyzed redox reaction is presented here. This investigation was carried out by a selected group of high school students. At the end of the experiment, the procedural and conceptual understanding of students was probed using specially designed multiple-choice questions (MCQs). Students' responses to MCQs and experimental readings reflected their skills such as understanding about appropriate controls, hands-on skills, and procedural understanding. Analysis indicated that 57% of the students demonstrated Lower Order Cognitive (LOC) skills while only 23% of the students demonstrated Higher Order Cognitive (HOC) skills. Students' responses indicated that their procedural understanding varied between 14 - 50%, while hands-on skills were in the range of 27 - 41%. This shows the need of such laboratory practices in regular biology curriculum. Inclusion of this experiment as a guided inquiry in the regular curriculum can help students enhance their HOC skills.
Keywords: *biochemical exercise, hands-on skills, HOC skills, LOC skills, procedural understanding, succinate dehydrogenase*
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INTRODUCTION

The rapid growth in knowledge in the discipline of biology necessitates a major shift in the approach towards teaching. The focus needs to shift from reciting of facts and knowing of terminologies to understanding concepts, acquiring skills to analyse and applying information to challenging new situations (Fiona, 2014). According to Bloom's taxonomy, observation and recall of facts as well as comparing and contrasting data are considered as Lower Order Cognitive (LOC) skills, while analysing and organising data, applying knowledge in a novel situation and generalisation from facts are considered as Higher Order Cognitive (HOC) skills (Krathwohl, 2002). Further, assessments that are designed to test students' above-mentioned HOC skills and competencies will force students to adopt a 'deeper approach' to learning as against the 'superficial approach' to learning (Momsen *et al*., 2013). The biochemistry experiment reported here was designed as an effort to assess the LOC and HOC skills as well as hands-on skills of students. Since this study was carried out on high-achieving high school students, it was used in a 'test' format. However, the same exercise can be used in a 'guided' format in a regular educational set-up so as to enhance the above-mentioned cognitive skills in students.

In the current high school curriculum, apart from the classical biology subjects, such as plant and animal anatomy, there is a major emphasis on understanding concepts in some areas of modern biology, such as molecular biology and biochemistry. Thus, biomolecules and cells and enzymatic processes form the core concepts in the high school biology course. It is expected that the hands-on activities performed in the laboratory during the course would further strengthen the concepts involved. It is feasible to handle various types of cells and perform experiments using biomolecules in the wet lab. However, when it comes to metabolic processes, students mostly rely on textbooks to learn the basic reactions. Also, several concepts, such as aerobic respiration, TCA cycle, and mitochondrial electron transport, are abstract and intangible to most students. Students' content learning can be improved in these areas if they get an opportunity to experiment with any of the reactions involved in these processes. Also, such experiments and related questionnaires, if designed appropriately, can help test students' hands-on skills as well as conceptual and procedural understanding.

There have been some reports describing experiments to test and analyse these skills in students' conceptual understanding in the laboratory (Vartak *et al.*, 2013; Latourelle *et al.*, 2019) as well as in the field (Dresner *et al.*, 2014). As part of a project carried out at our Centre, our major objective is to develop innovative experimental tasks in various areas of biology so as to nurture as well as test the HOC skills in high school students.

Here we present the results of an experimental test along with the subsequent questionnaire that was designed to probe students' above-mentioned skills.

METHODOLOGY

Subjects

The subjects in this study were a group of 35 students from different high schools all across India. They were all high achievers (top 1% meritorious students in biology) in their respective classes in the routinely conducted tests. They participated in a biochemical experiment which had been developed by the authors.

Materials

Laboratory Manual for Students (Box 1, shown on p. 4 – p. 5)

Multiple–choice Questionnaire (Box 2, shown on p. 6)

All materials mentioned in the Laboratory Manual for Students (Box 1) were prepared by the instructor beforehand. All reagents used were of analytical grade.

Methods

The participating students were asked to carry out the biochemical experiment independently by following the instructions of the laboratory manual (Box 1). After performing the biochemical experiment, they were asked to answer the questionnaire (Box 2).

INTERPRETATION OF THE BIOCHEMICAL EXPERIMENT

The experiment described in Box 1 involves electron transport chain observed *in vivo* in aerobic respiration. Using mitochondrial membrane systems from liver cells, the flow of electrons is studied. However, the path of electrons is blocked in the complex IV by the addition of NaN_3 in the reaction mixture and providing an external electron acceptor, dichlorophenolindophenol (DCPIP). This results in the change in the colour of reaction mixture as a result of the reduction of DCPIP. Thus Set 5 indicates the specific enzyme reaction involving succinic acid as a substrate, succinate dehydrogenase as the enzyme, and DCPIP as the terminal electron acceptor molecule. Sets 1 – 4 serve as controls. Set 1 shows a small reduction in the optical density (OD) value in spite of the absence of the specific substrate which is succinic acid.

Box 1: Laboratory Manual for Students

Biochemical Experiment on Succinate Dehydrogenase

Introduction to the Experiment:

Among the various enzymes involved in aerobic respiration, succinate dehydrogenase is unique as it is linked to both TCA cycle and electron transport chain (White, 2004). This enzyme catalyses both the oxidation of succinate to fumarate and the reduction of FAD to $FADH_2$. The electrons then travel through the components of the electron transport chain to the terminal electron acceptor, which is oxygen in case of aerobic respiration (Figure below).

The reaction that takes place in intact mitochondria can also be studied *in vitro* using different compounds as electron acceptor molecules. To study the activity of succinate dehydrogenase *in vitro,* dichlorophenolindophenol (DCPIP) has been used as an electron acceptor in this experiment. This compound is blue when oxidized and colourless when reduced. To divert the flow of electrons from Complex III & Complex IV to DCPIP, sodium azide (NaN_3) which acts as an inhibitor of cytochrome a_3 in Complex IV (See Figure below) needs to be added.

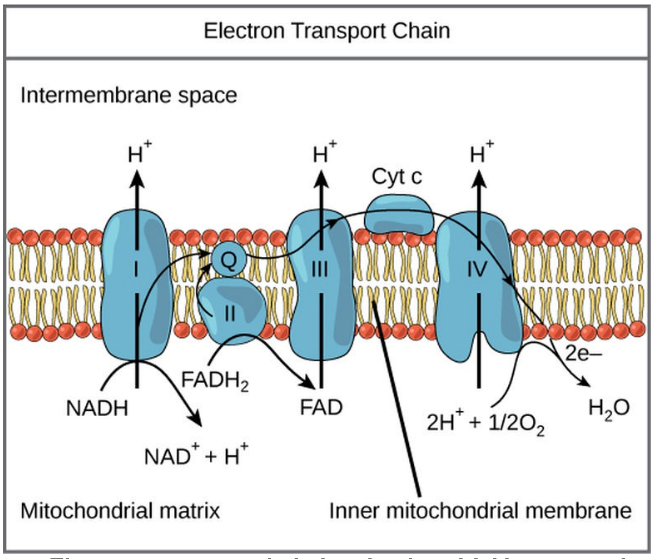

Figure: Electron transport chain in mitochondrial inner membrane.
(Source: https://commons.wikimedia.org/wiki/File:Figure_07_04_01.jpg)

Materials and Methods:

Freshly obtained chicken liver extract, Potassium phosphate buffer pH 7 (0.5 M), Succinic acid solution (0.15 M), DCPIP solution (1.5 mM), NaN_3 solution (0.2 M), Cuvettes, Micropipettes, Colorimeter, Test tube stand

Preparation of Chicken Liver Extract: Cold conditions (4°C) need to be maintained throughout the preparation of extract until use in the experiment. 2.5 g of freshly obtained clean frozen chicken liver is cut into small pieces and transferred into a mortar. 10 ml of cold phosphate buffer is added and the tissue is macerated well using a pestle to prepare a homogenous crude extract. The extract is transferred to a tube and centrifugation is carried out at 10,000 rpm for 10 minutes. The supernatant obtained is stored at 4°C till use.

Experimental Procedure:

In this experiment, the activity of succinate dehydrogenase is studied over a period of 30 minutes. Prepare the five sets of tubes for the experiment as given in Table. The entire experiment is carried out at room temperature (28°C). Instead of test tubes, 5 ml capacity cuvettes (diameter 1 cm), which can be inserted directly into the colorimeter to read the optical density (OD) values at 620 nm, are used. Thus, for each set, the reaction can be carried out in the same respective tube throughout.

Table: Experimental Protocol

Set No.	Phosphate buffer (I)	Succinic acid (II)	Sodium azide (III)	DCPIP (IV)	OD 620 nm	Liver extract	OD 620 nm				
					Baseline reading*		0 min	5 min	10 min	15 min	30 min
1	2.6 ml	-	0.1 ml	0.1 ml		50 µl					
2	2.5 ml	0.1 ml	0.1 ml	0.1 ml		-					
3	2.6 ml	0.1 ml	0.1 ml	-		50 µl					
4	2.6 ml	0.1 ml	0.1 ml	-		-					
5	2.5 ml	0.1 ml	0.1 ml	0.1 ml		50 µl					

**Baseline reading refers to the OD values for each tube after mixing reagents I to IV as given in the table.*

Readings:

At the time mentioned in Table (0 min to 30 min), read and record the OD value at 620 nm of each cuvette.

This indicates that the extract being crude is likely to have some reducing equivalents in a small quantity which reduce DCPIP slightly. Set 2 indicates the stability of the electron donor and also takes care of any reaction that takes place between the electron donor, the substrate, and NaN_3. Set 3 takes care of any OD due to turbidity of the liver extract which can then be subtracted from the actual reading obtained in Set 5. The mixture of solutions in Set 4 is colourless and transparent and the OD is expected to remain close to zero throughout the experiment. The OD values of this set indicate that succinic acid, NaN_3 and phosphate buffer do not react with each other to give any change in OD.

RESULTS AND INTERPRETATION OF STUDENTS' PERFORMANCE

Performing the experiment in the given format allows us to test students' hands-on skills. The skills can be broadly divided into handling of glassware, pipetting of small volumes of liquids, and handling of instrument. The OD values obtained by an expert (Table 1) can be used as a reference. Baseline readings of Sets 1, 2 and 5 are good indicators of students' pipetting skills. Consistent and comparable values (compared to the values in Table 1) obtained by the students indicate accuracy in pipetting. Also, OD values obtained for each set are indicative of handling of the instrument, namely, the colorimeter. OD values less than 50% of the expected values would be the result of incorrect selection of wavelength, improper blanking, and/or errors in pipetting. If the students have obtained large negative values (- 0.05 onwards), it indicates that no proper blanking has been done by the student. Figure 1 shows that only 41% and 27% of students exhibited accurate pipetting and instrument handling skills, respectively.

In the questionnaire, Question 1 deals with analysis of five possible sources of the enzyme with focus on their locations in the cell and roles in aerobic or anaerobic respiration. It thus tests students' abilities, such as comprehension of text, comparing and contrasting the facts as well as predicting outcomes, which are considered as LOC skills. Fifty-seven percent of the students could answer it correctly (Figure 2). Question 2 deals with students' understanding of the processes that are underway in the five experimental sets followed by extrapolating the results to a new experimental set-up. Having understood the path of

Table 1: Data obtained by an expert

Set No.	OD 620 nm					
	Baseline reading	0 min	5 min	10 min	15 min	30 min
1	0.75	0.69	0.64	0.63	0.63	0.64
2	0.74	0.72	0.71	0.70	0.71	0.70
3	0.01	0.06	0.06	0.06	0.06	0.06
4	0.01	0.01	0.01	0.01	0.01	0.01
5	0.72	0.70	0.58	0.49	0.42	0.34

electrons in normal electron transport chain and the effect of NaN_3 on it, students are expected to speculate the effect of omitting it in the experiment. This requires HOC skills such as application of a concept to a new situation and only 23% of the students exhibited this skill (Figure 2).

Box 2: Multiple-choice questionnaire

Questionnaire

Q. 1. Which of the following preparations will be a good alternative source of succinate dehydrogenase?

a. Cell membrane fraction of any aerobically respiring tissue.
b. Nuclear fraction of cardiac tissue.
c. Microsomal fraction of a tissue from secretory gland.
d. Crude cell lysate of bacteria isolated from a deep wound.
e. Crude extract of macerated muscle tissue.

Q.2. An experimenter added the 6th set with the following composition of solutions:

Set No.	Phosphate buffer	Succinic acid	Sodium azide	DCPIP	Liver extract
6	2.5 ml	0.1 ml	-	0.1 ml	50 µl

What result can be expected from this set?

a. OD lower than Set 1 but higher than Set 5
b. OD lower than Set 2 but higher than Set 1
c. OD lower than Set 5 but higher than Set 3
d. OD very close to zero reading
e. OD lower than Set 1 but higher than Set 3

Q. 3. Which set indicates the non-specific reduction of the electron acceptor?

a. Set 1
b. Set 2
c. Set 3
d. Set 4
e. Set 5

Q. 4. How can one obtain the value of non-specific reduction of the electron acceptor?

a. OD of Set 2 – OD of Set 1
b. OD of Set 5 – OD of Set 3
c. OD of Set 1 – OD of Set 5
d. OD of Set 2 – OD of Set 5
e. OD of Set 5 – OD of Set 4

Q. 5. Which set is indicative of the non-specific OD of the liver extract?

a. Set 1
b. Set 2
c. Set 3
d. Set 4
e. Set 5

Q. 6. Which of the sets indicates that the oxidized form of the electron acceptor is stable under the experimental conditions?

a. Set 1
b. Set 2
c. Set 3
d. Set 4
e. Set 5

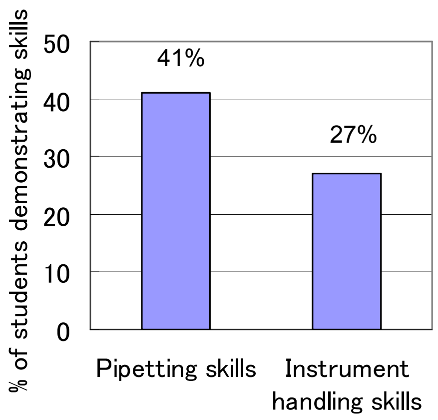

Figure 1: Students' accuracy in hands-on skills

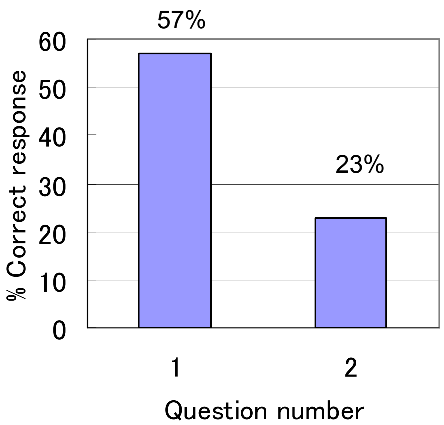

Figure 2: Students' responses to Questions 1 and 2 assessing LOC and HOC skills respectively

Procedural understanding is an important aspect of science process skills (Gott and Duggan, 1995; Roberts, 2001) and involves understanding about the rationale of the protocol being followed. In the present investigation, a reaction involving succinate dehydrogenase is underway in Set 5 while the other four sets represent different types of controls that are essential. Questions 3 – 6 probe students' understanding about the importance of appropriate controls in the experiment. This is a part of procedural understanding and as is evident from Figure 3, maximally up to 50% students exhibited this skill.

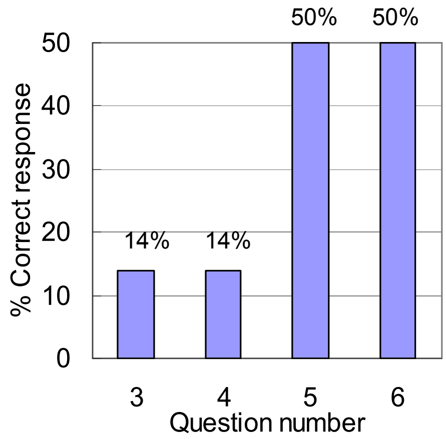

Figure 3: Students' responses to Questions 3 – 6 assessing procedural understanding

CONCLUDING REMARKS

It has long been realised that assessment reforms are greatly needed in higher education. Well–designed theory tests can help probe students' conceptual understanding, ability to interpret data and graphs as well as other cognitive skills. However, a well-designed laboratory exercise can additionally test students' hands-on skills and procedural understanding. Performing experiments in the laboratory takes students very close to a researcher's mind and allows them to actively participate in the process of collecting and analysing data. Also applying the acquired knowledge to novel situations can further strengthen students' HOC skills.

The experiment presented here is of a short duration and also does not require any sophisticated instrument or expensive chemical. The analysis shows that although the participating students were high achievers in the biology course, they were not very successful in getting practical (experimental) skills. This shows that there is a need to strengthen hands-on skills and procedural understanding in teaching regular biology courses at the high school level. Although this exercise was used as a testing module, the same module can be used as a learning tool for students if conducted as a guided inquiry session by a facilitator in the laboratory.

ACKNOWLEDGEMENTS

The authors acknowledge the support of the Department of Atomic Energy, Govt. of India, under Project Identification No. RTI4001.

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Practical Report

Use of "Enzyme Powder" for Inquiry Activity on Starch Digestion in Elementary School Science

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(Received: 01 December 2020; Accepted for publication: 14 July 2021;
Communicating editor: S. Mayama)

In Japan, elementary school children have been using their own saliva for experiments on starch digestion when they learn about the digestion and absorption of food in science. In carrying out these experiments, the use of their own saliva is particularly important for pupils, because they can notice that their own digestive juices digest food inside their digestive organs. However, various germs may be contained in saliva, so that considerable caution is required to avoid infectious diseases. In addition, pupils dislike providing their saliva for these experiments. To overcome such obstacles, we have introduced a step, the preparation of saliva enzyme powder by the cold ethanol precipitation method, into the experimental procedure. Since the ethanol precipitation technique is too advanced for pupils, this step must be done by the teacher as a demonstration of the method. In our trial of the laboratory class on starch digestion, at first, a solution of the saliva enzyme powder, which the teacher had prepared, and a sheet of starch-containing paper were used to confirm that the saliva enzyme powder could digest starch. Then, pupils were given a question, "Do living things other than human beings also contain digestive aids to digest starch?" To find an answer to the question, pupils examined whether starch digestion would occur by the crude enzyme powders from germinating corn grains and "kome-koji" (malted rice with *Aspergillus* sp.) which were prepared by the same method as the saliva enzyme powder. In their experiment, small polythene bags and plastic straws were used instead of test tubes and syringes, respectively, to reduce the cost of the experiment. More than four fifths of pupils expressed affirmative impressions of the laboratory class.

Keywords: *α-amylase, elementary school science, inquiry activity, saliva powder, starch digestion*

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INTRODUCTION

In elementary school science in Japan, "degradation of starch by saliva" has been the most familiar experiment in learning about the digestion and absorption of food. It is particularly important for pupils to carry out this experiment by using their own saliva, because they can notice that their own enzymes digest food inside their digestive organs. In the experiment, one of two methods for collecting saliva, "direct (spit) method" or "swab absorbing method," is commonly adopted. In the former method, the donor spits into a vessel. In the latter method, the donor holds a cotton swab in his mouth to absorb saliva. Kroen (1998) has already mentioned that college students rebel against spitting into tubes. Masamoto and Serita (2007) reported university students expressed less feeling of repulsion toward the swab-absorbing method. However, according to our preliminary investigation with a questionnaire, most elementary school children disliked both methods, because they did not want the other pupils to watch them spiting or to see their saliva. In addition, various germs may be contained in saliva, so that considerable caution is required to avoid infectious diseases (Shmaefsky, 1990). Therefore, many teachers have not put this experiment into practice in their science classes.

To overcome such obstacles, Kroen (1998) used α-amylase and amyloglucosidase commercially extracted from a bacterium and a fungus, respectively, by Sigma Chemical Company. In the present study, we invented a way to use the crude enzyme powder of saliva prepared by the cold ethanol precipitation method, which is commonly applied to the preparation of macromolecular compounds such as proteins and nucleic acids (*e.g.*, Kaga and Arai, 2004). In addition, we developed laboratory exercises on starch digestive enzymes to encourage pupils' inquiring minds. The laboratory exercises include activities to observe the degradation of starch in boiled rice grains by the crude enzyme powders of germinating corn grains and of "kome-koji" (rice malted with a mould, *Aspergillus* sp.) prepared by the same method as the saliva enzyme powder. In the present paper, we explain the methods for the preparation of crude enzyme powders and the procedures of pupil laboratory exercises, and report on a classroom trial of the exercises.

PREPARATION OF CRUDE ENZYME POWDERS

Materials

All materials used in the present study are easily available at stores in Japan. A packet of freeze-dried kome-koji (Figure 1), corn grains, and a bottle of absolute ethanol are sold at a grocery store, gardening shop, and pharmacy, respectively.

Preparation of the crude enzyme powder of saliva

After cleaning the inside of the mouth, a clean absorbent cotton ball was put in the mouth to absorb saliva. Then, the cotton ball was transferred into a small zippered polythene bag. This operation was repeated several times and the saliva was squeezed from the cotton balls in the polythene bag. The saliva collected was dripped into a beaker containing 100 cm^3 of cold absolute ethanol. Then, the beaker was kept in a refrigerator overnight until the precipitate accumulates at the bottom. The supernatant was removed gently from the beaker, and the wet precipitate was transferred into a Petri dish and kept in a refrigerator until it dried completely. The dried precipitate (hereafter we called it "saliva enzyme powder") was wrapped in a sheet of paraffin paper and kept in a small bottle with a desiccant. The bottle was kept in a refrigerator.

Preparation of the crude enzyme powder of germinating corn grains

Corn grains (Figure 2A) were soaked in water overnight (Figure 2B) and sown in a flowerpot. The flowerpot was placed under a room light. When the seedlings grew to about 5 cm in height, the sprouting corn grains were harvested and washed (Figure 2C and 2D). The leaves and roots were removed from each sprout, and the rest (scutellum and endosperm parts within the grain coat, Figure 2E) was kept in a freezer.

Figure 1: Packet of kome-koji

The frozen material was ground with a mortar and pestle to make a paste, and 50 cm^3 of cold water was added to the paste (Figure 3A). After being stirred well, the mixture was filtered by a tea strainer and the filtrate was refrigerated (Figure 3B). After the filtrate separated into precipitate and supernatant (Figure 3C), the supernatant was dripped into a beaker containing 100 cm^3 of cold absolute ethanol (Figure 3D). After this, the procedure for obtaining the crude enzyme powder of germinating corn grains (hereafter we call it "germinating corn enzyme powder") was the same as that for obtaining the saliva enzyme powder (Figure 3E).

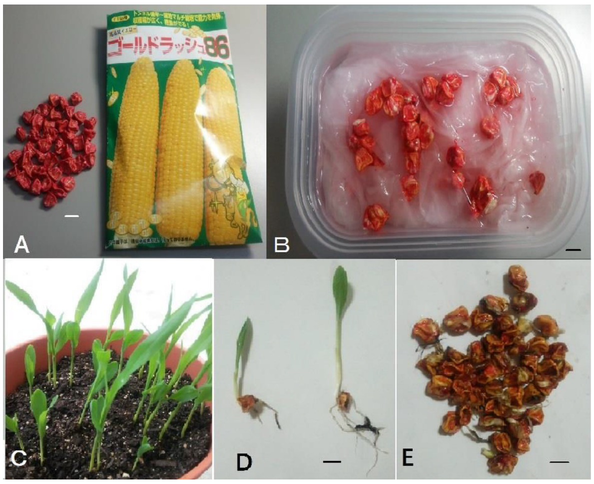

Figure 2: Preparation of germinating corn grains
A: Corn grains (germicide-coated), B: Corn grains soaked in water overnight, C: Corn seedlings seven days after being sown, D: Harvested corn seedlings, E: Germinating corn grains, from which leaves and roots were removed.

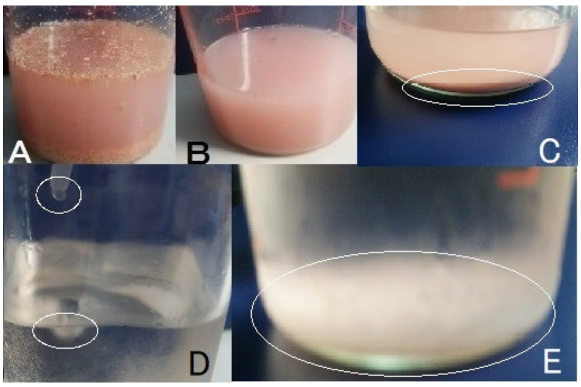

Figure 3: Preparation of germinating corn enzyme powder
A: Suspension of ground germinating corn grains, B: Filtered suspension C: Starch precipitating at the bottom (circled), D: Supernatant being dripped into cold ethanol (circled), E: Crude enzyme precipitation accumulating at the bottom (circled).

Preparation of the crude enzyme powder of kome-koji

Ten grams of freeze-dried kome-koji and 100 cm^3 of water were mixed in a beaker and the mixture was stirred well. The beaker was kept in a refrigerator until the suspension separated into precipitate and supernatant (Figure 4A). The supernatant was dripped into a beaker containing 100 cm^3 of cold absolute ethanol (Figure 4B). After this, the procedure for obtaining the crude enzyme powder of kome-koji (hereafter we call it "kome-koji enzyme powder") was the same as that for obtaining the saliva enzyme powder (Figure 4C).

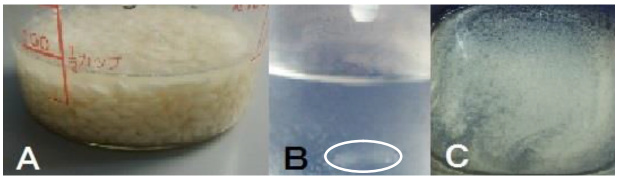

Figure 4: Preparation of "kome-koji enzyme powder"
A: Kome-koji suspended in water, B: Supernatant dripped into cold ethanol (circled), C: Crude enzyme precipitation accumulating at the bottom.

PROTOCOLS FOR LABORATORY EXERCISES

Table 1 shows the protocols for pupil's laboratory exercises on starch digestion which we tested at an elementary school.

TRIAL OF PUPIL LABORATORY EXERCISES

Preparation of materials

Enzyme powders: Although it is preferable that pupils carry out all steps, we prepared all enzyme powders beforehand, and at the start of the exercises we demonstrated the way of pre-

Table 1: Protocols for pupil's laboratory exercises

= Protocols for Laboratory Exercises on Starch Digestion =
Materials Enzyme solutions (saliva, germinating corn, kome-koji, digestive aid) A gargle containing iodine A cotton swab, a sheet of starch-containing paper, a paint brush Five boiled rice grains, five small zippered polythene bags, six transparent plastic straws, and a polystyrene (Styrofoam) bowl with warm water of about 40ºC for each group
Experiment 1: Observation of starch digestion in starch-containing paper 1) Absorb saliva enzyme solution by a cotton swab. 2) By means of the cotton swab, write some letters on a sheet of paper. 3) After a few minutes, apply a diluted gargle solution to the paper with a paint brush.
Experiment 2: Observation of boiled rice grain digestion by various enzyme solutions 1) Write "saliva," "corn," "kome-koji," "digestive aid," or "water" on each of the five polythene bags. 2) Into each polythene bag, put one boiled rice grain and pour about 1 cm^3 of water. 3) Zip the bags firmly, and then, rub the bags to mash the boiled rice grains thoroughly. 4) Into each of the first four bags, pour about 1 cm^3 of corresponding enzyme solution. 5) Into the "water" bag, which is used as a control, pour about 1 cm^3 of water. 6) Zip the bags firmly and rub them to mix their contents well. 7) Soak these bags in warm water and leave them for a while. 8) Pour about 1 cm^3 of diluted gargle solution into each bag and observe the colour of the mixture in each bag.

paring saliva enzyme powder. Major reasons for doing the preparation as a demonstration are that absolute ethanol is flammable and its vapor is unsafe for pupils to handle. The demonstration is indispensable, because if the teacher uses an enzyme powder obtained by the cold ethanol precipitation method without demonstrating this preparation process, pupils may misunderstand that the powder is a kind of digestive aid.

Enzyme solutions: A small amount of each enzyme powder (saliva, germinating corn, and kome-koji) and digestive aid was dissolved into water. Prior to the class, we confirmed that each enzyme solution had enough activity to digest a boiled rice grain completely within 10 min.

Digestive aid (a powder/granulated one) and a gargle containing iodine (or Lugol's solution) were purchased at a pharmacy. The gargle, of which iodine content is 0.7%, was diluted into 10% for Experiment 1 and 1% for Experiment 2 with water.

A sheet of paper was confirmed to include starch beforehand, because some sorts of paper contain a synthetic paste instead of starch.

Plastic straws of 6 mm in diameter were used. These were marked at 3.5 cm from an end to indicate the volume of about 1 cm^3. By immersing this end in a liquid to the mark and blocking up the other end with a fingertip, a 1 cm^3 liquid can be taken.

Experiment 1

Although it is preferable that pupils carry out all experiments, this experiment was conducted by the teacher as a demonstration, because of time limitations. At first, the teacher led pupils to have a group discussion on what they had learnt about the working of saliva in the previous class through the textbook. Then, he explained an outline of Experiment 1 and that the paper to be used included starch and led pupils to predict the result of the experiment.

This experiment could allow pupils to ascertain the working of saliva and the method of detecting it.

Experiment 2

Experiment 2 was carried out by the pupils as a group activity. At first, the teacher posed a question, "Do living things other than human beings also contain digestive aids to digest starch?". To find the answer, pupils were advised to mull over whether starch digestion would occur in plants and moulds. Then, they were introduced to Experiment 2 where the enzyme solutions of a plant (germinating corn grains) and a mould (kome-koji) were used. A saliva enzyme solution and a solution of digestive aid were also used as comparatives.

Pupils were asked to predict the results of the experiment. Then, they carried out the experiment by following the protocols.

Results

In Experiment 1, a few minutes after writing letters, the saliva enzyme solution dried and the traces of letters left wrinkles in the paper (Figure 5A). When the diluted gargle solution was applied, the paper was dyed bluish purple except the parts where the letters had been written with the saliva enzyme solution remained colourless (Figure 5B). The result allowed pupils to understand that saliva can digest starch.

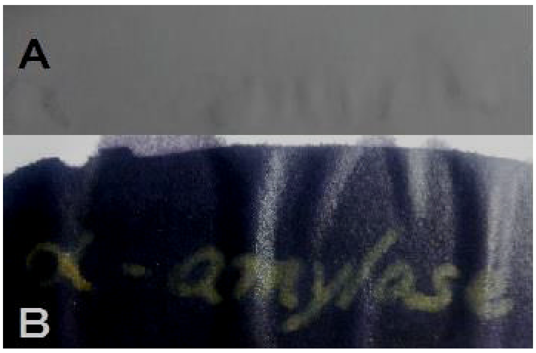

Figure 5: Starch digestion in starch-containing paper

A: Starch-containing paper with letters written in saliva enzyme solution, B: Same paper with diluted gargle solution applied.

In Experiment 2, by rubbing the bags in step 3), the boiled rice grain in each bag was mashed into tiny fragments suspended in liquid. In step 8), when the diluted iodine solution was poured in, the mixtures in the bags marked "saliva," "corn," "kome-koji," and "digestive aid" remained colourless, while that in the "water" bag turned bluish purple (Figure 6). The results indicate that, along with saliva and digestive aid, the crude enzyme powders of plants and moulds can digest starch.

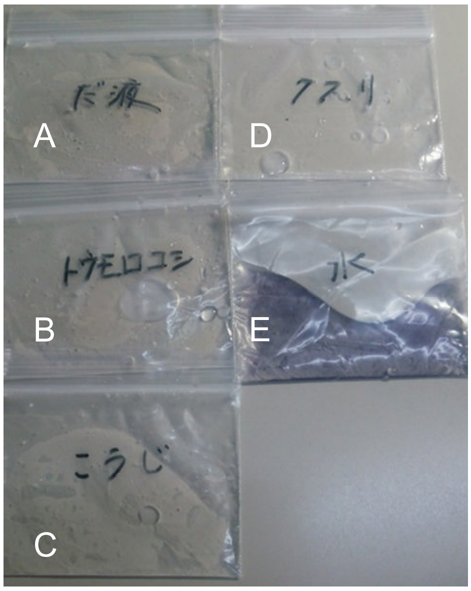

Figure 6: Digestion of boiled rice grains by various enzymes

A: Saliva, B: Germinating corn grain, C: Kome-koji, D: Digestive aid, E: Water (control)

DISCUSSION

Saliva (salivary amylase) has commonly been used in student laboratory exercises on carbohydrate (starch) digestion (*e.g.,* Germann, 1991). The use of saliva is simple, easy, and cheap, but as mentioned in the Introduction, there are two issues in using human saliva that should be surmounted. Freeland (1974) and Wyatt (1974) proposed the use of the digestive organ of a freshly killed animal, but schoolchildren may feel this to be repulsive. As proposed in this report, the cold ethanol precipitation method is applicable not only for preparing saliva enzyme powder but also for preparing crude enzyme powders of various living materials. By the ethanol treatment, any living germs can probably be eliminated from enzyme preparations.

It is well-known that the activity of saliva amylase varies considerably with individual, and even with the same person the activity is changeable dependent on body conditions. This is the same with the other living materials. Crude enzyme powders prepared by the cold ethanol precipitation method from various organisms can be kept in a refrigerator for a long time. Once teachers determine a suitable amount of these powders to be dissolved in water, they can use them without examining it as the need arises.

The use of a sheet of starch-containing paper for detecting starch digestive enzyme(s) is convenient and practically costless, so that this is suitable for any school setting, even those in developing countries. For Experiment 2, teachers can also use the paper, but we recommend using a starch-containing staple food in each country. Brown (1994) in the U.S.A. used McMush in her student laboratory to analyze the organic compounds found in an ordinary fast-food lunch. In Asian countries, boiled rice is more familiar to schoolchildren than McMush, easily available, and almost costless. Furthermore, the use of small polythene bags and plastic straws instead of test tubes and syringes, respectively, can also reduce the cost of the experiment.

To determine the activities of starch digestive enzymes, Davis (1977) used the radial diffusion method. As his method is suitable for a quantitative assay of the amylolytic enzymes and it takes much time to prepare the agar disks for the experiment, it would be better to introduce it into biology laboratories at the secondary level. Laboratory exercises proposed by Hogue (1972) and Law (1996) are also suitable for student laboratories at the secondary level to deepen student understanding of digestion and absorption in our digestive organs, because the procedures of these experiments seem to be somewhat complicated and unsuitable for schoolchildren.

The latest Course of Study for Elementary Schools and its guidelines were announced in 2017 by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT; See its website, also refer to Asia Society website). The purpose of the subject “Science” mentioned in the Course of Study is “to allow children to foster their talents and abilities for solving questions about natural things and phenomena scientifically.” For achieving the purpose, schoolteachers are asked to let pupils get close to nature, to make the most of the scientific way of observing and thinking, to carry out an observation or an experiment with insight, etc. In addition, pupils are expected to nurture the feeling of love toward nature, to understand natural things and natural phenomena, to acquire basic skills of observation and experimentation, to foster the ability of problem-solving, and to develop (independent) positive attitudes toward problem-solving. In our classroom trial, more than four fifths of pupils expressed affirmative impressions of the laboratory activity. Examples of pupil comments are as follows:

“I am glad I could carry out hands-on laboratory activities on the working of saliva;”

“Through the observation and experimentation, I can easily understand what I have not understood.”

However, to know what skills and what knowledge pupils can achieve through the inquiry activities we have proposed, further investigations are required. Germann (1991) proposed DIAL (SPS)2, the Directed Inquiry Approach to Learning Science Process Skills and Scientific Problem

Solving, which can be adopted to student laboratories possibly at secondary and tertiary levels, to help students develop problem-solving skills. With directed inquiry activities as introduced in the present paper at the primary level, pupils can acquire basic science processing skills and some background knowledge which is required for proceeding to the further inquiry activities which Germann (1991) described.

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Practical Note

Improving the First-Year Biology Educational Experience for Japanese Students at an American University Campus

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(Received: 25 June 2021; Accepted for publication: 09 October 2021)

Keywords: *Biology and Culture, Diversity, Intercultural education*

INTRODUCTION

Interaction between the United States and Japan is essential for the scientific and technological growth of both regions. Communication and sharing of innovation is a two-way street, with both countries dependent on each other for markets, research, education, and defense; in spite of this, the education of Japanese students of biological sciences in America can be very problematic. As with any academic subject, there can be problems with the English language that are difficult to overcome. Even with students arriving in America with good language skills, there are problems specific to biology coursework that can be ameliorated by application of specific pedagologic techniques.

BACKGROUND

Northeastern State University of Oklahoma (NSUOK) is in an American four-year university in the central part of the United States. Historically it was a college of the Cherokee Nation that was unique for the large percentage of Native Americans in the student population. NSUOK enrolls a large number of Japanese *ryūgakusei*, *i.e.*, overseas students, in the science departments. First-year biology students are enrolled in a course called "General Biological Science". This course serves as the entry point for biology majors and includes an introduction to the origins of living organisms and the mechanisms of evolution that gave rise to the current diversity of species. It also covers the origins and characteristics of major groups in the three domains of living organisms. The subject matter for the first four weeks is indicated in Table 1. Students arriving in America from Japan would have been introduced to these topics as part of the secondary school education in Unit One of Advanced Biology of the Japanese National Curriculum Standard Course of Study (Nakamichi and Katayama, 2018).

We administered a 12-item survey (See Appendix) to students in the fifth week of the

Table 1: Content of the First-Year General Biological Science curriculum at Northeastern State University of Oklahoma

General Biological Science
Lecture Topics

Week 1:	Biology – the Study of Life Basic Chemistry of Cells
Week 2:	Organic Molecules of Cells Structure and Function of Cells
Week 3:	Dynamic Activities of Cells
Week 4:	Pathways of Photosynthesis Pathways of Cellular Respiration

first-year biology course and then stratified the results of the Japanese versus the American students in order to get some insight into the educational process in relation to the overseas students. The survey instrument was based on a survey previously used for Japanese nursing students with permission of the authors (Cox and Yamaguchi, 2010). Students were also allowed to write in comments on the survey.

RESULTS

Two hundred and twenty-three responses were scored on a seven-point Likert scale, and the results of the most significant four items are summarized in Figure 1.

The statements showing the major discrepancies between the American and Japanese students are all related to the style of instruction or the dynamics of the classroom environment. The Japanese students rated the quality of instruction lower in all measured categories. Japanese students also expressed discomfort with small group work in groups of four to five students. Following these discussion groups, one individual is selected to present the results to the entire class. While the Japanese students would often have a mastery of the material and adequate language skills, they would often sit back and defer to the American students within their group for the leadership in these assignments. Interviews with students and biology faculty indicated that language ability was not a limiting factor, but that most difficulties were related to teaching style and classroom culture.

DISCUSSION

Understanding the science of biology requires an appreciation of all life on the planet, all continents as well as the sea. This is an international endeavor, so it is important to train biologists to work in overseas assignments, as well as to bring experience gained abroad back

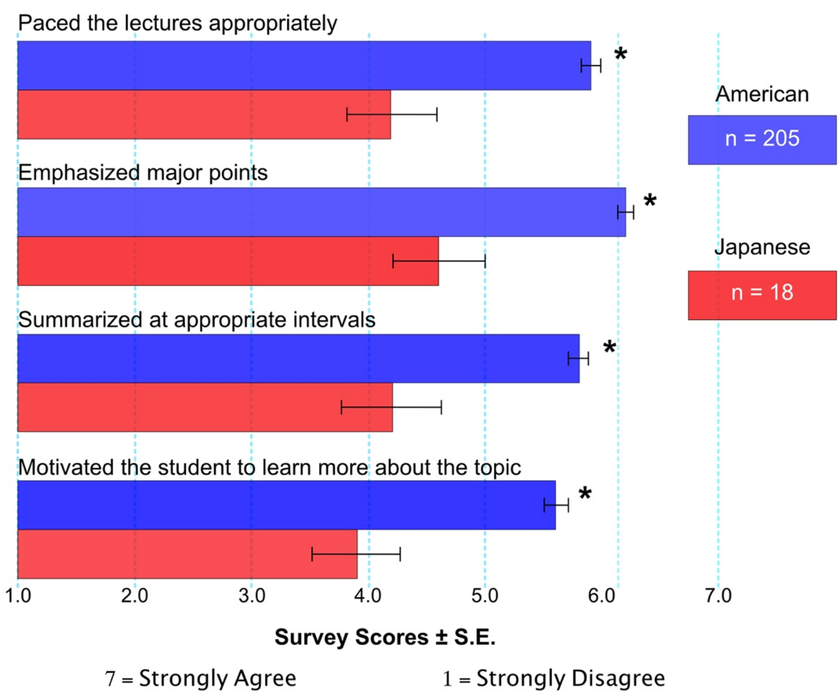

Figure 1: Results showing significant differences of four factors. The statements of the English language version are listed in the table, but statements were also provided in Japanese language. *p<0.05

to their home countries. A large percentage of biological science graduate students in the US are International, often 25 to 35% at major universities. The majority of these students are coming from Asian countries, so biological education in America certainly has an effect on the status of biology in Asia. Students primarily come from China, South Korea and India, but Japan continues to rank in the top eight places of origin (*Open Doors Report on International Educational Exchange,* 2020).

The problems faced by Japanese biology students are often related to culture rather than language ability (Hwang and Roth, 2008). Biology itself is something that may be foreign and strange, *i.e.*, the language of biology is often incomprehensible to the average American speaker of English. In order to teach science, professors must translate the language of science into the vernacular of the students. By reference to common ideas and experiences in everyday life, a professor can make abstract ideas into tangible concepts that serve as the building blocks of the students' knowledge. Much of the material that an American professor would use for real-life examples is laden with cultural values that are not easily interpreted by non-English speakers, even those with a substantial level of English proficiency.

OUR PLAN FOR IMPROVEMENT

Introductory biology courses at American universities are often large with 30-70 students. It is difficult for the professor to customize the course for the benefit of the few non-native students. Our intent involved improving integration of Japanese students while concurrently administering a survey Japanese and American students to better understand the dynamics involved for future refinement of the process. From our survey we determined that maintaining motivation was a main disparity between American and Japanese students, Figure 1. Our results show that the problems with this cohort of international students are related to cultural issues rather than language problems or lack of scientific background. Our approach was to concentrate on motivation and acculturation, expecting that this would concurrently improve the other identified problem areas of pacing, emphasis and recapitulation. Many approaches have been adopted to increase motivation in biology coursework including conducting laboratory experiments and research projects (Kaga and Arai, 2004), a "Meet a microbiologist" program (dela Cruz *et al.*, 2013), and conducting field-based observation (Sato *et al.*, 2004).

Our approach at NSUOK attempted to combine the best attributes of these programs into activities benefitting students who are new to the United States. Our approach centered on acculturation and included the following activities.

- Students were adopted by a biology graduate student who acted as a mentor.
- Students came to their mentor's research lab and were allowed to participate.
- Students attended the weekly lab meetings with primary investigator, graduate students, and international undergraduate biology students (Figure 2).
- Students shadowed their graduate student mentor during off-campus employment in a

Figure 2: Weekly lab meeting.

local hospital.

Benefits: The time spent with their mentor walking across campus from the classroom to the lab provided an opportunity to discuss the biological topics of the day in what would otherwise be considered downtime. This gave the graduate students additional teaching experience and understanding of the difficult parts of the day's lesson. The students felt freedom to speak up and discuss their difficulties, which they were often hesitant to do in the crowded classroom. The Japanese students often expressed a desire to socialize outside of their Japanese cohort, and they appreciated this opportunity.

Difficulties: Students participating in this program were required to complete additional laboratory safety training requirements of the university. Their limited time and experience did not allow them to complete their own research project during this time. The credentialing process to act as observers at the local hospital was prolonged, although not overly burdensome.

Summary: Our survey indicated that cultural factors are important for Asian biology students studying in America. Although program participants as well as non-participants were able to successfully complete their four-year biology degree in America and return to successful employment in Japan, post-graduation interviews indicated that participants saw the program as a helpful and rewarding experience directly contributing to their educational success. We believe this may serve as a model for other biology courses delivered to students with mixed cultural backgrounds, Japanese, as well as other foreign students.

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Note: The research portion of this study was approved by the IRB of NSUOK study number 101-11.

APPENDIX

The twelve points surveyed are listed below.

The points discussed in this paper are indicated in bold type.

1. Previewed main points of lecture
2. Organized content logically
3. **Paced the lecture appropriately**
4. **Emphasized major points**
5. Gave adequate examples to clarify major points
6. Adapted content to student's level of comprehension
7. **Summarized at appropriate intervals**
8. Made the content interesting
9. **Motivated student to learn more about the topic**
10. Used visual aids effectively
11. Used instructional aids effectively
12. Provided opportunities for questions

Biological Resource

Immobilized *Euglena* Cells (*Euglena* Beads) are Useful for Laboratory Exercises on Photosynthesis at the Secondary Level

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(Received: 01 December 2020; Accepted for publication: 25 November 2021; Communicating editor: K. H. Chu)

Euglena is a common photosynthetic protist, useful as teaching material in biology at secondary and tertiary levels, because it has some unique features of interest to students. *Euglena* can be cultured in an inorganic medium (HYPONEX solution) without contamination by heterotrophic microorganisms, rendering it suitable for experiments on photosynthesis. In this study, we immobilized *Euglena* cells in calcium alginate gel beads ("*Euglena* beads"), facilitating their repeated use in experiments on photosynthesis. In this immobilized state, *Euglena* could propagate could reproduce in 0.1% HYPONEX solution and gradually turned green under the culture conditions. The *Eugena* beads were demonstrated to be a useful substitute for aquatic plants, such as *Elodea* and *Cabomba*, in the qualitative experiments in which a pH indicator was used to detect photosynthetic CO_2 consumption. *Euglena* beads cultured for 30 days had sufficient photosynthetic activity to allow measurement of photosynthetic rates within 30 minutes.

Keywords: calcium alginate gel beads, Euglena*, immobilization, laboratory exercise, photosynthesis, secondary school biology.*

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INTRODUCTION

As land dwellers, we are familiar with terrestrial plants as photosynthetic organisms and, at primary and secondary levels, photosynthesis is usually taught using them as examples. At the secondary level, even when students study photosynthesis in aquatic ecosystems, aquatic plants, such as *Elodea* and *Cabomba* (Crawford, 2005; Adams *et al*., 2012), have usually been employed. However, in aquatic ecosystems algae are also important primary producers (Sze, 1997). Therefore, practical classes using algae are important for secondary students in understanding global ecosystems. Hull (1966) indicated that cultured unicellular microalgae can be used in laboratory exercises of plant physiology, including photosynthesis. However, in practice, this has not often been adopted.

Usually, the isolation and pure culture of microalgae is demanding for student experiments, and a convenient method for culturing microalgae in the school laboratory setting (*e.g.*, Hull, 1966) would be valuable. However, most species are not easy to be cultured, with *Euglena* being an exception for which simple methods have been developed (*e.g.*, Flinn Scientific, see Website list). Koizumi and Mikami (1976) proposed a simplified culture method for *Euglena*, using only HYPONEX powder, which is available at any gardening shop. *Euglena* is a common photosynthetic protist with some well-known unique features making it useful as teaching material in biology. It is now familiar to people because it has been used as a nutrient supplement, as an ingredient in foods, and for producing biodiesel and jet fuel. Therefore, the use of this alga for biology laboratory exercises may stimulate students' interest.

In this study, we aimed to use *Euglena* to inform students that not only higher aquatic plants, but also microalgae, are major producers in freshwater ecosystems. However, there are problems with using *Euglena* cell suspension for detecting/measuring photosynthesis. A major problem is that when cell suspensions are used, it is difficult to harvest the cells for reuse, meaning that we must prepare a large amount of *Euglena* suspension for each experiment. To solve this problem, we introduced an immobilization technique, which allows us to use the same *Euglena* cells repeatedly. Tamponnet *et al.* (1985) showed that *Euglena* cells immobilized in a calcium alginate matrix maintained photosynthetic activity and ultrastructural integrity. After immobilizing *Euglena* cells in calcium alginate gel beads, we examined whether the immobilized *Euglena* could reproduce and whether such "*Euglena* beads" could be used for the qualitative and quantitative experiments commonly carried out in secondary schools in Japan.

MATERIALS AND METHODS

Euglena *cell culture*

For culturing *Euglena* cells, we used a 0.1% solution of HYPONEX powder (N:K:P = 6:10:5, HYPONEX Corporation), hereafter referred as "Hyponex medium" as described by Koizumi and Mikami (1976) (See Note[1]). Cells of *Euglena gracilis* Klebs from Mikami's laboratory at the Miyagi University of Education, were cultured in flasks containing 250 cm^3 Hyponex medium in an incubator at 20°C under a light intensity of 40 - 50 $\mu mol/m^2/s$ with a 12:12 h light:dark photoperiod. At 7-week intervals, a 2.5 cm^3 cell suspension was inoculated into 250 cm^3 of fresh Hyponex medium.

Immobilization of Euglena *cells and culturing* Euglena *beads*

Euglena cells were immobilized by Matsuda's (1994) method. To 120 cm^3 of mature (7-week-old) cell suspension, 1.5 g of sodium alginate (Wako Pure Chemicals) was added, and the solution was stirred gently until the powders were dissolved completely. It was dripped from a pipette (bore size approximately 2 mm diameter) into cool 5% calcium chloride solution with gentle stirring. As a result, about 1800 *Euglena* beads were made from 120 cm^3 of mixture. After standing in 5% calcium chloride solution for 30 minutes, the *Euglena* beads were washed three times with distilled water. The beads were then put into flasks containing 200 cm^3 of Hyponex medium and cultured in a incubator.

After a day, they were transferred to fresh Hyponex medium including 0.03 M $KHCO_3$ and exposed to high intensity light (200 $\mu mol/m^2/s$) for two hours in order to promote photosynthesis, and then transferred to 200 cm^3 of fresh Hyponex medium before returned to the incubator. This high-intensity-light treatment was repeated every 10 days during the experiment.

Calcium alginate gel beads lacking *Euglena* cells, but otherwise identical, were used as a control.

Qualitative experiment on photosynthesis using Euglena *beads*

In qualitative experiments on photosynthesis commonly carried out in junior high school science classes in Japan (Yamazaki and Tahara, 1998), the *Euglena* beads were used instead of water plants. Bromothymol blue (BTB), a pH indicator, was used for detecting photosynthetic CO_2 consumption (See Note[2]). To 200 cm^3 of 1 mM $KHCO_3$ solution, 5 cm^3 of BTB stock solution (0.04 w/v%) was added. At this point, the color of this alkaline mixture solution was blue. Then, exhaled air was blown in for about 30 seconds until the mixture turned green ("BTB solution").

About 300 *Euglena* beads or control beads, cultured for more than 30 days, were washed twice with BTB solution and kept in 200 cm^3 BTB solution for about two hours to equalize the pH inside the beads. The beads were then transferred into respective 20 cm^3 vials of BTB solution and the vials sealed, with care to avoid air bubbles. For each of three samples, the *Euglena* beads, the control beads, and the blank test (BTB solution only), three vials were placed in high-wall Petri dishes with 20°C water, placed on an OHP (FUJIX EZ-2) and illuminated for 60 minutes. Sheets of paraffin paper between the Petri dishes and the OHP glass deck were adjusted to light intensity of 300 - 350 $\mu mol/m^2/s$ (Fig. 1). The vials were lightly shaken by hand once every five minutes. The color of the BTB solution was observed at 15-minute intervals by placing the vials on white paper.

Measurements of the photosynthetic and respiration rates of* <u>Euglena</u> *beads

The photosynthetic O_2 production and the respiratory O_2 consumption of cultured *Euglena* beads were measured with the Productmeter (Yokohama *et al.*, 1980). The amount of O_2 was calculated according to the method of Yokohama *et al.* (1986).

About 150 each of the *Euglena* beads and the control beads were put into the reaction vessel and the compensation vessel of the Productmeter, respectively. To each vessel, 7.5 cm^3 of Hyponex medium and 0.5 cm^3 of 0.5 M $KHCO_3$ solution were added, giving a final concentration of $KHCO_3$ of 0.03 M. The vessels of the Productmeter were immersed in a water bath with temperature controlled by a circulator (COOLNIT CL-150F, TAITEC) and mechanically shaken continuously. The light source was a slide projector (S-300, ELMO), with the intensity adjusted by measuring the photon flux density with a photometer (MEMORY SENSOR MES-101, Koito Industry).

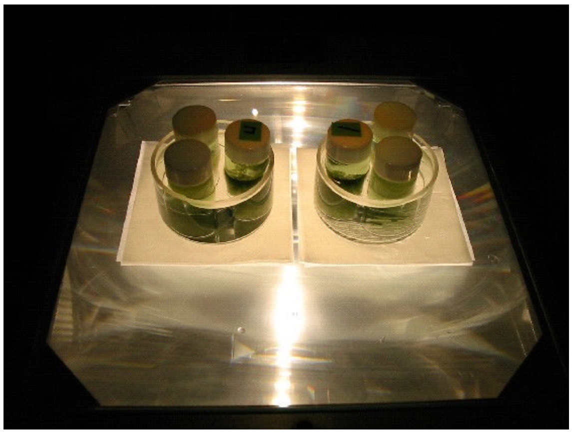

Figure 1: Vials immersed in 20°C water and illuminated by an OHP

After each measurement, the *Euglena* beads and the control beads were washed twice with fresh Hyponex medium prior to reuse. After a series of measurements, the beads were put into flasks containing 200 cm^3 of Hyponex medium and cultured in the incubator until required for further experiments.

RESULTS

<u>Euglena</u> *can reproduce in an immobilized state*

Under the culture conditions, *Euglena* beads (Fig. 2) gradually turned green. Thirty days after starting the culture, their green color became deeper (Fig. 3). Microscopic observation confirmed that the cells had formed many colonies (Figs. 4 and 5). The beads did not disintegrate at least for two months, and few *Euglena* cells were lost from the beads during the culture period.

Qualitative experiment on photosynthesis using* <u>Euglena</u> *beads

Figure 6 shows *Euglena* beads (left), control beads (center) and a blank test (right). Under illumination, the color of the BTB solution in the

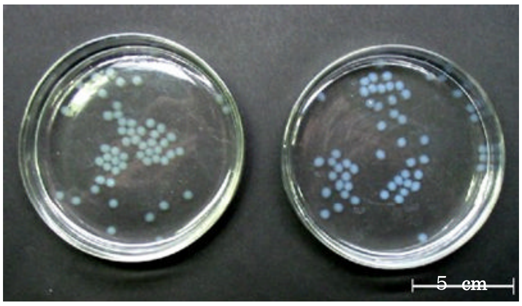

Figure 2:* <u>Euglena</u> *beads (left) and control beads (right) before culturing

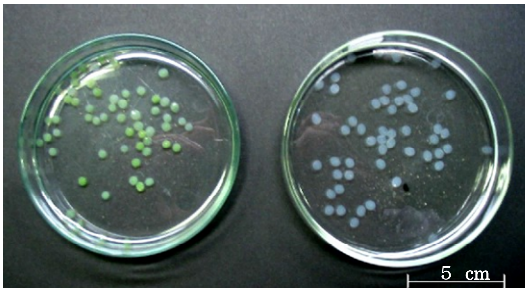

Figure 3:* <u>Euglena</u> *beads (left) and control beads (right) cultured for 30 days

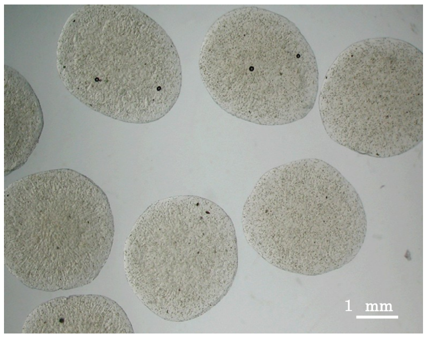

Figure 4: A micrograph of* <u>Euglena</u> *beads before culturing (×10)

Some black globules seen in the beads are bubbles. The *Euglena* cells are smaller brown grains.

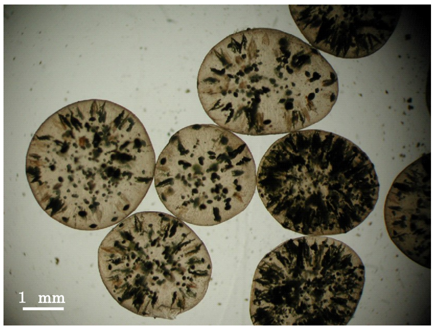

Figure 5: A micrograph of* <u>Euglena</u> *beads cultured for 30 days (×10)

Many colonies of *Euglena* cells are formed in the gel matrix of the beads.

vial containing the *Euglena* beads changed from green to blue-green within 30 minutes (Fig. 6B), and to blue within 45 minutes (Fig. 6C). Control beads and the blank did not change color. The change from green to blue indicates the consumption of dissolved CO_2 in the BTB solution (See Note[2]), confirming that the *Euglena* cells in the beads consumed CO_2 through photosynthesis.

Changes in the photosynthetic oxygen production of* <u>Euglena</u> *beads during culturing

The change in the photosynthetic oxygen production of *Euglena* beads (about 150 beads) in culture was examined. Initially, the photosynthetic oxygen production was 10.0±3.9 μl O_2/h. Twenty days later it increased by almost eight times. Furthermore, the photosynthetic oxygen production of 30-day-old *Euglena* beads was nearly two times higher than that of 20-day-old

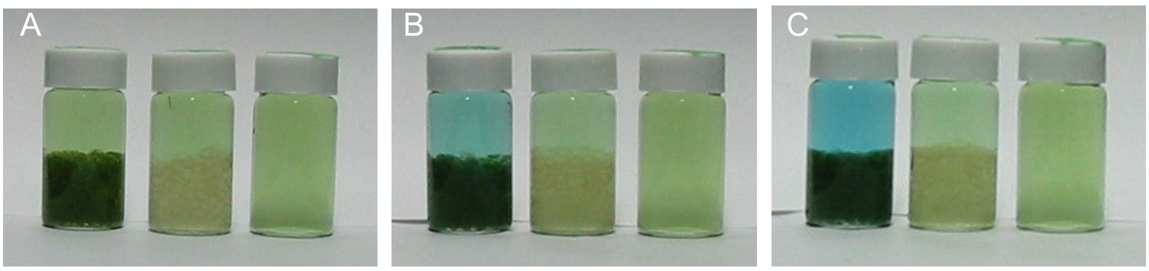

Figure 6: Changes in the color of the BTB solution

A: At the beginning, B: Under light illumination for 30 minutes, C: Under light illumination for 45 minutes. *Euglena* beads are on the left, control beads are in the center, and a blank test is on the right.

ones (Fig. 7). This increase in oxygen production in culture resulted from the reproduction of *Euglena* cells in the gel beads.

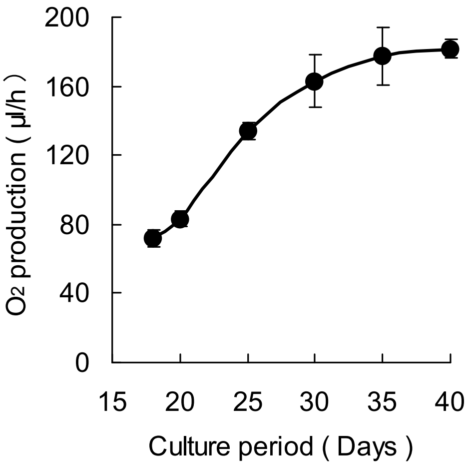

Figure 7: Changes in the photosynthetic oxygen production of <u>Euglena</u> beads in culture

The measurements were done under the light intensity of 200 μmol/m^2/s at 30°C. The *Euglena* beads (approx. 150) were used repeatedly for measurement.

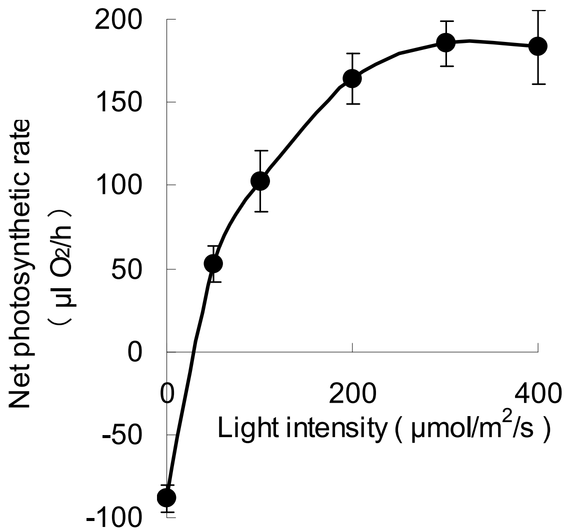

Figure 8: Photosynthesis-light curve of <u>Euglena</u> beads

The measurements were carried out at 30 ºC. The *Euglena* beads (approx. 150) were used repeatedly for measurement.

Photosynthetic curve of <u>Euglena</u> beads

The photosynthesis-light curve (Fig. 8) and the photosynthesis-temperature and respiration-temperature curves (Fig. 9) were obtained by using 30-day-old *Euglena* beads. Light saturation at 30°C occurred at around 300 μmol/m^2/s (Fig. 8). The optimum temperature for photosynthesis at the light intensity of 200 μmol/m^2/s was at around 20 - 30°C (Fig. 9).

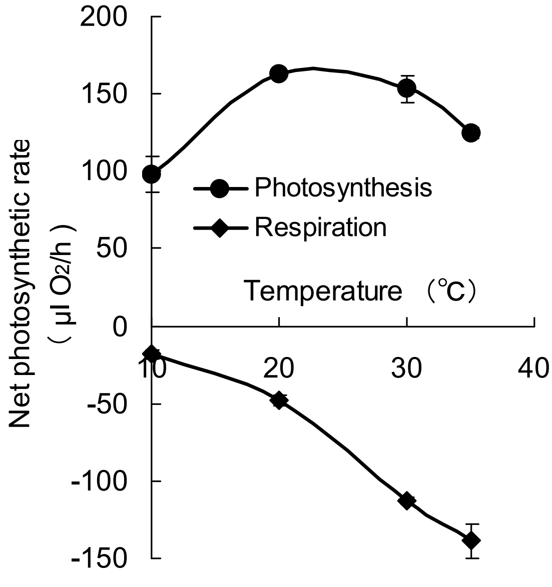

Figure 9: The photosynthesis-temperature curve and respiration-temperature curve of <u>Euglena</u> beads

The measurements of photosynthesis were carried out at a light intensity of 200 μmol/m^2/s. The measurements of respiration were carried out in dark conditions. The *Euglena* beads (approx. 150) were used repeatedly for measurement.

DISCUSSION

Euglena cells possess many features that make them useful in biology education. These include: 1) they can be observed under a low-power microscope; 2) they can swim by means of a flagellum and execute phototactic responses; 3) they can change cell shape; 4) they have chlorophyll *a* and *b*, like green plants, though its major photosynthetic product is not starch; 5) under inappropriate growth conditions they can encyst, while under appropriate growth conditions they can reproduce rapidly and form water-blooms; and 6) they sometimes accumulate huge amounts of carotenoid pigments and become reddish. Therefore, this microalga can be used for a variety of student exercises in biology, not only for microscopic observation, but also for experiments on phototaxis (Espey, see Website list) and population growth (Oswald and Kwiatkowski, 2011). It also has the potential to be used for environmental monitoring in environmental education (Danilov and Ekelund, 2001; Ahmed and Häder, 2010). However, no report on the use of this organism for a secondary student laboratory exercise on photosynthesis has been published. This is possibly because of its motility and tendency to be contaminated by bacteria in liquid culture (See Note[1]).

In the present study, to facilitate and simplify experiments on *Euglena* photosynthesis in secondary schools, *Euglena* cells were immobilized in calcium alginate beads as described by Matsuda (1994), a method similar to those of CAROLINA Biological Supplier Co. and SAPS: Science and Plants in School (See respective websites). Immobilization techniques of immotile microalgae were developed in the 1980s and are now used for a wide variety of biotechnological applications (Kaparapu, 2017). Some freshwater green algae, such as *Scenedesmus* (Chevalier and Noüe, 1985), *Botryococcus* (Bailliez *et al*., 1986) and *Chlorella* (Llangovan *et al*., 1998), and marine microalgae (Hertzberg and Jensen, 1989; Moreno-Garrido *et al.*, 2005) retain their photosynthetic activities in calcium alginate or κ-carrageenan beads. The use of microalgae (indefinite species) immobilized in calcium alginate beads, or "algal balls," in an experiment on photosynthesis in student laboratory classes has already been proposed (CAROLINA Biological Supplier Co. and SAPS). Similarly, Eldridge (2004) used immobilized *Scenedesmus quadricauda*, and Crawford (2006) and Andrews *et al.* (2015) followed Eldridge's method in their practical studies at schools.

In the present paper, we show that the immobilization seems effective for a motile microalga, such as *Euglena*, permitting uncomplicated measurement of photosynthetic activity in biology laboratory classes at the secondary level. Our results indicate the following three advantages for such studies: 1) the beads can be used for experiments repeatedly so a large quantity of culture does not need to be prepared; 2) the experiments can be done under different conditions with the use of the same beads; 3) in a qualitative experiment, photosynthesis can be observed simply by watching the color of a pH indicator solution.

In the qualitative experiment, the use of *Euglena* beads allows us to detect the changes in color of the BTB solution more easily in comparison with the use of free algal cells, because the *Euglena* cells are contained by the gel beads, and we can observe the color of the solution easily without any contamination of the *Euglena* cells (Fig. 6). When the *Euglena* beads cultured 30 days or more were used, the photosynthetic CO_2 consumption by *Euglena* cells could be detected within 30 minutes by examining the color of the BTB solution. So, this experiment can be carried out in one school hour of secondary schools.

In the quantitative experiment, when a 30-day-old *Euglena* beads culture was used, the amount of oxygen evolved from the *Euglena* beads is high enough for measuring their photosynthetic oxygen production with the Productmeter within 30 minutes under the conditions described above. Therefore, the experiment is possible to be accomplished within a one period of secondary schools.

In order to use the *Euglena* beads for these quantitative and qualitative experiments, we had to culture them for more than 20 days. If we could use an algal culture of high cell density for the immobilization procedure, we would be able to use the algal beads for photosynthesis experiments immediately after making them. However, we could not obtain such a high cell density because of the slow growth rate of *Euglena* cells under the culture conditions of Koizumi and Mikami (1976) suggested. Although immotile microalgae can be collected from suspension culture by low-speed centrifugation, we could not adopt this method for *Euglena* since the precipitated *Euglena* cells were dispersed into supernatant immediately. Therefore, further studies are required to test the concentration and the kind of HYPONEX for culture medium as well as the other culture conditions, such as temperature and light intensity.

Chevalier and Noüe (1985) used immobilized *Scenedesmus* cells for sewage treatment repeatedly in their laboratory tests. *Euglena* can reproduce well in eutrophic waters such as sewage and frequently lead to algal blooms, as does *Scenedesmus*. *Euglena* was reported to be useful for removing nutrients and heavy metals from sewage (Ahmed and Häder, 2010), so that immobilized *Euglena* cells can possibly be used for sewage treatment at municipal scale. In schools, the *Euglena* beads can be used as teaching material, not only in biology, but also in environmental education programs that focus on algal blooms caused by eutrophication and in studies of sewage treatment.

Note [1]: A standard culture method for *Euglena* is shown on the website of Flinn Scientific (*cf.* its website). The culture media mentioned in this website are not difficult to prepare, but the preparation of Hyponex medium is much easier. We, however, did not adopt the culture media provided by Flinn Scientific, because these culture media contain organic substances and, on the website, bacterial contamination is mentioned. When we adopt such culture media, it always requires a strict sterilization practice to avoid bacterial contamination. Yet such practice is often difficult in the general biology laboratory setting in secondary schools. If bacterial contamination occurs, we cannot measure the correct photosynthetic rate and respiration rate of *Euglena* beads.

Note [2]: At around pH 7, the color of a diluted BTB solution is green. It turns yellow in acid conditions and blue in alkaline. When a BTB stock solution is mixed with 1 mM $KHCO_3$ solution (an alkaline solution), the color of this mixture is blue. When exhaled air is blown into the mixture, CO_2 in the exhaled air dissolves into the mixture, thereby acidifying the mixture and turning its color green. As the dissolved CO_2 in the BTB solution is consumed by photosynthesis, this raises the pH of the solution gradually and changes its color back to blue. The detectable pH range of BTB is 5.6 – 8.2, and some other pH indicators which have a similar detectable range can be substituted for BTB (*cf.* SAPS website).

ACKNOWLEDGEMENT

We are very grateful to Professor Emeritus Kazuyuki Mikami of the Miyagi University of Education, who provided us with cultured *Euglena* cells and gave us valuable advice on culturing *Euglena*, and to Professor Emeritus Peter Tyler of Deakin University, Australia, who helped us to edit the manuscript.

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CAROLINA Biological Supplier Co.: Using algae beads as a model for photosynthesis
https://www.carolina.com/teacher-resources/Interactive/essentials-algae-beads/tr40904.tr <accessed: Oct. 20, 2021>

Flinn Scientific: Culturing *Euglena*
https://www.flinnsci.com/culturing-euglena/dc10578/ <accessed: Oct. 20, 2021>

SAPS (Science and Plants in School): Photosynthesis with algal balls
http://www.saps.org.uk/secondary/teaching-resources/235?dm_i=19FH,189MY,6Q22VI,45OSG,1 <accessed: Oct. 20, 2021>

Espey, M: *Euglena*, the phototaxic protozoa (Winning Experiment Procedures from the NIH LAB Challenge)
https://www.drugabuse.gov/euglena-phototaxic-protozoa <accessed: Oct. 20, 2021>

Publications

Biology Education for Social and Sustainable Development (ISBN: 978-94-6091-925-1) was published in 2012 by Sense Publishers, Rotterdam, Netherlands (http://www.sensepublishers.com/). Some papers presented at **the 23rd Biennial Conference of the AABE** which was held in Singapore in October 2010 were compiled in this book by Dr. Mijung Kim and Dr. C. H. Diong. You can refer to the abstracts of these papers in **the sixth volume of the *Asian Journal of Biology Education*** (2012).

Biology Education and Research in a Changing Planet (2015) (ISBN 978-981-287-523-5) was published by Springer (http://www.springer.com/in/book/9789812875235). Some papers presented at **the 25th Biennial Conference of the AABE** which was held in Malaysia in October 2014 were compiled in this book by Dr. Esther Gnanamalar Sarojini A Daniel. The abstracts of these papers were included in **the eighth volume of the *Asian Journal of Biology Education*** (2015).

From the Editor-in-Chief

The COVID19 pandemic, which started in early 2020, still continues in 2021. It forces us to lead a restricted daily life. AABE activities are also affected by the pandemic; particularly, its biennial conference has to be postponed. The 28th Biennial Conference of the AABE (AABE28) was planned to be held in Tianjin, China, in October 2020, but we have not been able to hold it even in this year, 2021.

Under such severe circumstances, I am very pleased we can publish the thirteenth volume of the *Asian Journal of Biology Education* (AJBE) in this year. In this volume, there are two practical reports, one practical note, and one report on biological resources, though it cannot include the AABE28 conference report.

During last two years, the manuscripts contributed to AJBE have been peer-reviewed by the following persons as well as the Editorial Board members: Professor Nobuaki Asakura (Kanagawa University, Japan), Mrs. Teiko Nakamichi (Tokyo Institute of Biology Education, Japan), Dr. Danny Ng (The Chinese University of Hong Kong, China), and Dr. Takayuki Sato (Hirosaki University, Japan). I deeply appreciate their efforts to review the manuscripts critically.

The next volume of the AJBE will include a special issue related to education on the COVID19 pandemic. The expected topics are education on infectious diseases, microbes/viruses, immunology, etc. Everyone can contribute their research papers and/or practical reports on these topics to AJBE. General topics on biology education and environmental education are also welcomed. So, I would like to ask the AJBE readers to prepare their manuscripts referring to the "Instructions to Contributors" and send them to me.

Dr. Nobuyasu Katayama (katayama@u-gakugei.ac.jp)

Appendix

Symbols and Abbreviations for AJBE

TERM / UNIT OF MEASUREMENT	SYMBOL / ABBREVIATION
altitude	alt.
ampere	A
ångström (0.1 µm)	Å
calorie	cal
candela	cd
centimeter	cm
circa (Latin: approximately)	*ca.* or *ca*
cubic centimeter	cm^3
cubic meter	m^3
cubic millimeter	mm^3
day	d
degree Celsius	ºC
degrees of freedom	df *or* *v*
et alia (Latin: and others)	*et al.*
et cetera (Latin: and others)	*etc.*
exempli gratia (Latin: for example)	*e.g.*
figure, figures	Fig., Figs.
gram	g
greater (more) than …	>
hectare	ha
hertz (frequency)	Hz
hour	h
id est (Latin: that is)	*i.e.*
joule	J
Kelvin	K
kilo-	k
kilocalorie	kcal
kilogram	kg
kilometer	km
latitude	lat.
less than …	<
liter	l *or* L
lumen	lm
lux	lx
maximum	max.
meter	m
micro-	µ
microgram	µg
microliter	µl *or* µL
micrometer	µm
milli-	m
milligram	mg
milliliter	ml *or* mL
millimeter	mm
minimum	min.
minute (time)	min
molar (concentration)	M, mol L^{-} *or* mol/L
mole	mol
month	mo
nano-	n
nanometer	nm
not (statistically) significant	NS *or* ns
number	No.
number (sample size) =	*n* =
parts per million	ppm
percent	%
plus or minus	±
probability	*p*
revolutions per minute	rpm
second (time)	s
species (singular / plural)	sp. / spp.
square centimeter	cm^2
square meter	m^2
square millimeter	mm^2
standard deviation	SD
versus	vs. *or* v.
volume	vol.
volt	V
watt	W
week	wk
weight	wt
year	y