JOURNAL OF CHEMICAL EDUCATION

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Laboratory Experiment

Integration of Teaching Laboratory Activities Based on the Valorization of Industrial Waste into Chemical Education to Address the Emerging Sustainable Development Goals

Published as part of Journal of Chemical Education special issue "Action for Climate Empowerment in Chemistry Education".

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Cite This: *J. Chem. Educ.* 2024, 101, 4405−4410

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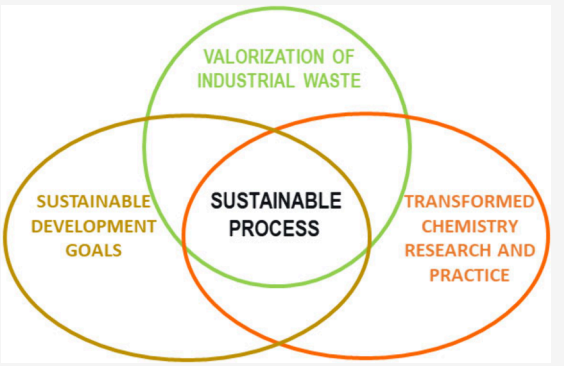
ABSTRACT: The integration of learning strategies based on the valorization of industrial waste into education is crucial for students to raise awareness of climate change causes and to thrive in the emerging circular bioeconomy. An attractive laboratory experiment focused on the production of second generation bioethanol from apple pomace is proposed. With this approach, undergraduate students of technological specialties explore real routes for the valorization of industrial food wastes. The activity allows them to become aware of the current energy outlook, the causes of climate change, and Sustainable Development Goals (SDGs) of the 2030 Agenda. Moreover, the proposal facilitates acquisition of chemical, technological, and mathematical knowledge and incorporation of important skills and competencies in future professional activities. In this way, students will promote social changes that guarantee the protection of the environment and improve the quality of life, in line with the Education for Sustainable Development (ESD).



KEYWORDS: *Undergraduate Students, Laboratory Instruction, Problem Solving/Decision Making, Collaborative/Cooperative Learning, Student-Centered Learning, Laboratory Equipment/Apparatus, Hands-On Learning, Industrial Chemistry, Sustainability*

INTRODUCTION

On September 25, 2015, the General Assembly of the United Nations (UN) adopted the 2030 Agenda for Sustainable Development. The Sustainable Development Goals (SDGs) include the adoption of urgent measures to combat climate change and its effects, in oceans, forests, and lands.[1−3] The UN emphasizes the importance of Education for Sustainable Development (ESD) in the SDGs4 Quality Education, which includes target 4.7: "*By 2030, ensure that all learners acquire the knowledge and skills needed to promote sustainable development, including, among others, through education for sustainable development and sustainable lifestyles...*".

The higher educational system is a great ally in working toward the SDGs, promoting real and effective changes to achieve global social well-being and sustainable lifestyles.[4] Through education, it is possible to meet the knowledge, skills, and behaviors necessary to change the destiny of humanity, based on social and environmental development centered on attitudes of respect and care for others and their surroundings.[5] In this context, the role of "Environmental Technology", which applies scientific and engineering principles to study and improve the environment,[6] is key since it integrates Chemistry, Process Engineering, and Biotechnology areas.

The integration of SDGs in learning requires the proposal of new educational models in line with the ESD,[7] the reform of undergraduate curriculum,[8] and the incorporation of new competences[9] and concepts including climate change, sustainable development, waste upgrading, circular bioeconomy, industrial symbiosis, or "zero waste" industries, in line with biomass biorefineries. In this context, the search for innovative and attractive laboratory experiences in the fields of Chemistry, Environmental Technology, etc., with students at the center of learning, that increase the motivation and develop the capacity for reflection and critical thinking, while improving teamwork skills, is necessary.

According to Food and Agriculture Organization (FAO), close to one-third of the food produced worldwide is thrown away, which represents more than 1300 million tons of food

Received: June 14, 2024
Revised: August 15, 2024
Accepted: August 30, 2024
Published: September 19, 2024

https://doi.org/10.1021/acs.jchemed.4c00729
J. Chem. Educ. 2024, 101, 4405−4410

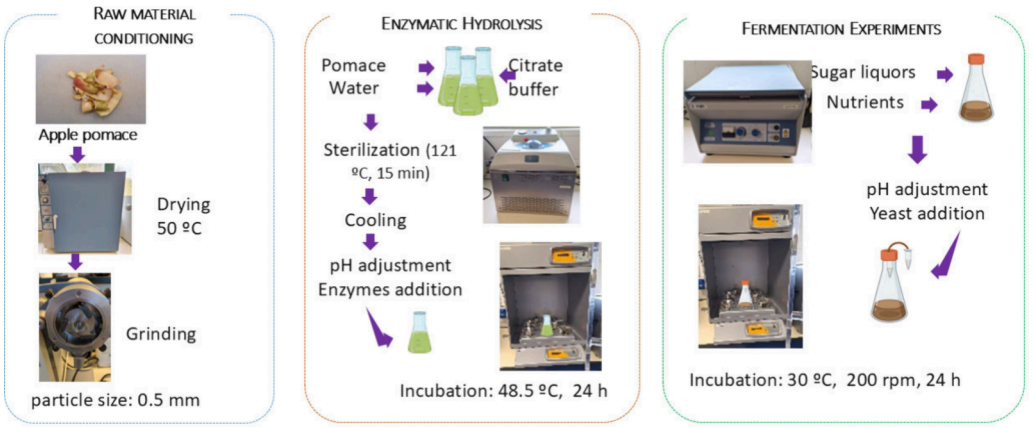


Figure 1. Experimental procedure.

waste and more than 900 million tons of fruit and vegetables.[10] Although both target 12.3 of the SDGs and current legislation aim to reduce food waste by 2030 and increase recycling and valorization routes, many of these wastes currently lack high value-added applications.[11] Food wastes are considered as a source of carbohydrates, minerals, fatty acids, phenolics, fiber, and protein. Due to their chemical composition, they have great potential for application in food, fuels, and chemical products.[11−13]

Second generation biofuels are obtained from several types of cellulosic nonfood biomaterials, including biomass and biowaste. Therefore, conversely to first generation biofuels, second generation fuels do not compete with food crops, neither for land nor for resources such as water,[14] and they can help to reduce greenhouse gases emissions associated with fuel oil consumption to combat climate change and mitigate environmental damage.[15] Bioethanol is a well-known renewable biofuel with interesting energetic value, low toxicity, low boiling point, and high-octane.[16] The production of second generation biofuels, such as bioethanol, from industrial food waste, raw materials with zero or very low cost, requires the enzymatic hydrolysis of the substrate for the production of sugar solutions, followed by the ethanolic fermentation stage.[12]

Apple pomace is a byproduct derived from cider and juice making industrial processes, with a high potential for integrated biorefinery approaches focused on the production of pectin, lignin, and bioethanol,[17] among others. It is a suitable feedstock for biotechnology processes, since it is mainly made up of sugars and polysaccharides (close to 65 wt %).[13] Moreover, it presents interesting contents in protein, phosphorus (P), magnesium (Mg), iron (Fe), etc., which could fulfill some of the nutritional requirements of several microorganisms, thereby decreasing the production cost.[13]

To date, several teaching studies were focused on food waste valorization,[18−20] on green and sustainable chemistry, and on the SDGs application.[21−23] However, in order to advance toward the SDG4 and SDG12, it is necessary to provide training to students that allows them to develop sustainable second generation food-waste biorefineries focused on the production of high added value biomaterials, biochemicals, and/or biofuels, as proposed Kumar et al.,[24] Rico et al.,[12] or Borujeni et al.[17] In this context, not much literature is available on second generation bioethanol production from food wastes for teaching laboratories in higher education institutions. For example, McCance et al.[18] and Zhou et al.[19] developed biorefinery approaches for the pineapple wastes and corn cobs, respectively. Epstein et al.[20] evaluated the production of bioethanol for several sources: fruits, grains, and grass; Mascal and Scown[25] and van Seters et al.[26] evaluated paper-like materials for the same purpose. However, many of these experimental procedures required chemical pretreatments with strong acids and/or bases, undesirable compounds for student laboratories, and they do not include mathematical models to evaluate the kinetics of production of the key compounds.

This work focuses on the implementation of SDGs in laboratory experiments in the field of chemical education and related areas. In particular, it proposes the production of second generation bioethanol from apple pomace by sequential enzymatic hydrolysis and fermentation technology.

EXPERIMENTAL WORK DESCRIPTION

The 3-day laboratory experiment (2−3 h per session) was carried out with more than 50 students (in small groups of 2−3 people) obtaining a degree in Energy Engineering from the University of Vigo (period 2016−2021). The background information on the project, the theoretical outline of work, and the form of evaluation were previously made available to the students. Figure 1 displays a scheme of the experimental procedure.

Chronogram of Activities in Every Laboratory Session and Main Results Obtained

Session 1. The students received a short training about the main scientific databases, which allowed them to find relevant information about apple pomace and bioethanol production from food waste. Afterward, they started the experimental sessions.

First, apple pomace samples were conditioned. Second, the students estimated the apple pomace potential for the production of biofuels, calculating its potential sugar content based on its polysaccharide content, following eq 1:

$$SC_{pot}\,(g/L) = \frac{SC_n/C_{Est}\cdot\rho}{LSR + 1 - L} \quad (1)$$

where SC_{pot} is the potential sugar concentration, SC_n is the sum of polysaccharide content of raw material, C_{Est} is the stoichiometric factor, ρ is the density of the medium, LSR is the liquid to solid ratio, and L is the lignin content of the raw material.

Third, taking into account the stoichiometry of the chemical reaction of the process (see eq 2) and the material balance, the students calculated the maximum ethanol concentration (E_{max})

https://doi.org/10.1021/acs.jchemed.4c00729
J. Chem. Educ. 2024, 101, 4405−4410

that could theoretically be achieved, as well as the maximum CO_2 concentration.

$$C_6H_{12}O_6 \rightarrow 2C_2H_6O + 2CO_2 \quad (2)$$

The value determined for SC_{pot} was 56 g/L; the maximum ethanol calculated according to the stoichiometry of the reaction was 28 g/L, and the maximum CO_2 was 27 g/L.

Fourth, the students carried out the enzymatic hydrolysis stage in an orbital shaker with two commercial concentrates, 10 FPU/g dried solid of "Celluclast 1.5L" and 10 UI/g dried solid of "Novozym 188" (kindly supplied by Novozymes, Madrid, Spain). Samples were withdrawn at the desired times, boiled, and filtered before colorimetric analysis.

Fifth, in order to employ the dinitrosalicylic acid (DNS) method (additional information is provided in Supporting Information, SI) for the quantification of reducing sugars in the following sessions, they determined a calibration curve with standard solutions of D-glucose in the concentration interval between 0 and 1 g/L (see Figure 2). The equation determined was the following: Glucose (g/L) = 0.146 + 1.679 Absorbance.

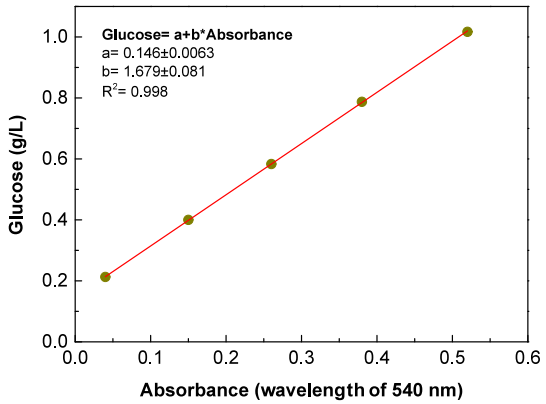


Figure 2. Calibration curve for glucose obtained by the DNS method.

In the event that it was necessary to reduce the duration of the practice, the calibration curve could be provided to the students. Alternatively, the sugar analysis could be performed by high performance liquid chromatography (HPLC).[12,13]

Sixth, they prepared *Saccharomyces cerevisiae* (a baker's yeast) inoculum in a culture medium containing 30 g/L glucose, 20 g/L peptone, and 10 g/L yeast extract.

Seventh, in this session, they also prepared the nutrient concentrate (on the order of 15 times).

Session 2. First, to carry out the alcoholic fermentation, the enzymatic hydrolysis liquors were introduced into a sterile Erlenmeyer flask along with the nutrients required by the yeast: 20 g/L peptone; 10 g/L yeast extract. During fermentation, the anaerobic conditions were guaranteed with the CO_2 produced, placing a perforated stopper with a CO_2 outlet connected to glycerol-locks to prevent the entry of O_2 in the medium. Next, the pH of the medium was adjusted to 6 and 10% (v/v) inoculum was added. Finally, the flasks were introduced into the orbital incubator. At desired times, samples were withdraw for the media and analyzed following spectrophotometric measurements: sugar analysis by the DNS method and the ethanol concentration by the "EnzytecTM Liquid Ethanol" from R-Biopharm, following the general instructions available on the Web site (https://food.r-biopharm.com).

Second, taking into account the calibration curve, the students were required to determine the sugar content of the samples obtained from the enzymatic hydrolysis assay by the DNS method. The kinetic obtained for the sugar concentration is depicted in Figure 3, where the degree of fit to the experimental

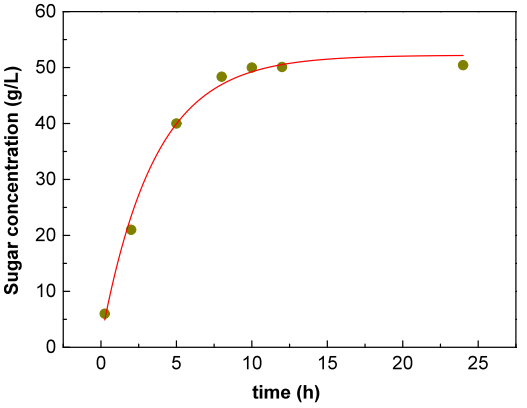


Figure 3. Example of experimental and calculated values determined for the sugar content of the enzymatic hydrolysis liquors.

and the calculated values by the Holtzapple model can also be seen:[27]

$$SC = SC_{MAX}\frac{t}{t + t_{1/2}} \quad (3)$$

where SC is the sugar concentration achieved at time t, SC_{MAX} is the maximum sugar concentration at an infinite reaction time, and $t_{1/2}$ is the time required to reach $SC = SC_{MAX}/2$, respectively. The time course of sugar concentration data is fit to the selected model by the least-squares method with $SC_{MAX} \leq SC_{POT}$. The values predicted for the Holtzapple model for SC_{MAX} and $t_{1/2}$ were 56.03 g/L and 2.07 h, respectively. The experimental sugar concentration obtained at 24 h was similar to the SC provided by the Holtzapple model, 50.43 and 51.58 g/L, respectively. The determined R^2 value was 0.9607.

Third, afterward, in order to obtain relevant information about the saccharification stage yield, the students should calculate the polysaccharide to sugar conversion (PSC) achieved in the enzymatic hydrolysis following eq 4

$$PSC\,(\%) = \frac{(SC_t - SC_0)}{SC_{pot}} \quad (4)$$

where SC_t and SC_0 are sugar concentrations at times t and 0, respectively, and SC_{pot} is the potential sugar concentration of the raw material. After 24 h of saccharification, the PSC reached close to 90%.

Alternatively, the sugar and ethanol analysis could be performed by HPLC,[12] thus reducing the time required for the experimental work. The ethanol content in the fermentation can be also followed by the CO_2 evolution determined by weighing the flasks,[11] by Turbidimetric Estimation,[28] or using the colorimetric oscillating reaction.[29]

Session 3. First, the students determined the sugar and ethanol contents of the different fermentation samples by colorimetric methods (see results in Figure 4).

Second, they fitted the ethanol kinetic to the modified Gompertz model, which predicted the maximum productivity, production, and the phase-lag time:[12,30]

https://doi.org/10.1021/acs.jchemed.4c00729
J. Chem. Educ. 2024, 101, 4405−4410

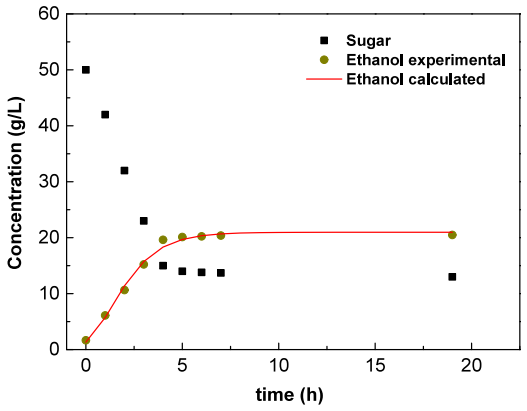


Figure 4. Example of experimental and calculated values determined for the sugar and ethanol content during the fermentation course by a group of students.

$$E_t\left(\frac{g}{L}\right) = E_{MAX}\cdot\exp\left[-\exp\left[\frac{EQ_{pmax}\cdot\exp(1)}{E_{MAX}}(t_{PL} - t) + 1\right]\right] \quad (5)$$

where E_{MAX} is the potential maximum ethanol concentration (g/L), EQ_{pmax} is the maximum productivity of ethanol (g/Lh), and t_{PL} is the phase-lag time (h).

The high degree of fit to the experimental concentration of ethanol and that predicted by the model can also be observed in Figure 4, where the experimental and the calculated values are included. The values determined by the modified Gompertz model for E_{MAX}, EQ_{pmax}, and the t_{PL} were 20.97 g/L, 5.81 g/Lh, and 0.02 h, respectively. The experimental ethanol concentration obtained at 24 h was really closed to the E_t provided by the modified Gompertz model, 20.49 and 20.97 g/L, respectively. Therefore, the model presented a good fit (R^2 = 0.9931).

Third, the students are required to calculate the ethanol yield and the ethanol volumetric productivity (EQ_p) of the fermentative stage following equations:

$$\text{Ethanol yield}\,(\%) = \frac{(E_t - E_0)}{0.51\cdot SC} \quad (6)$$

$$EQ_p\left(\frac{g}{L\cdot h}\right) = \frac{E_t}{t} \quad (7)$$

where E_t and E_0 are ethanol concentrations achieved at times t and 0, 0.51 is the stoichiometric factor for hexoses to ethanol conversion, and SC is the total sugar concentration of the fermentation media (g/L). As an example, the values obtained for the ethanol yield and the EQ_p at 19 h were 74% and 1.08 g/Lh, respectively.

Additional information concerning the laboratory activities is provided in the SI.

Main Objectives, Evaluation, and Student Assessment

The main goal of the learning experience is to introduce students to real approaches commonly used for the sustainable and environmentally friendly valorization of food wastes in a circular bioeconomy context as well as to strengthen stem professionals in modeling activities of great interest in process optimization.

At the end of the experiment, the students should be able to achieve the following learning objectives:

1. Students know the 2030 Agenda for Sustainable Development and the ESD as well as identify the SDGs implied in this work.
2. Students identify the high environmental impact of food waste and its great value as raw materials for the production of second generation biofuels.
3. Students raise awareness of the need of replacing fossil fuels by biofuels, thus reducing crude oil dependence and the emissions of greenhouse gases and helping in mitigating the heating of Earth and climate change.
4. Students acquire knowledge as well as chemical, technological, and mathematical skills for determining the main process variables and for modeling the sugar and bioethanol kinetics.
5. Students participate in classroom and increase their motivation, while improving teamwork skills and critical thinking, as well as other transversal competences highly demanded in the labor market.

At the end of laboratory sessions, the groups of students should draw up a report with the main results obtained (see the SI, the Template of the Report), including a discussion comparing them with available literature, present them in the classroom (15 days after finishing the experimental work), and answer some questions.

Students' performance was evaluated by the teacher based on a 4-point scale (Excellent: 4; Good: 3; Acceptable: 2; Poor: 1). The criteria used for the evaluation are depicted in Table 2 of the SI. The overall average for all criteria ranged from 3 to 4. The students yielded the lowest results in the strategy approach, outcome assessment, and the capacity to manage time. However, criteria associated with the work organization, the presentation and discussion of the results, and active involvement achieved excellent marks. It is worthwhile pointing out that the evaluation of learning goals associated with the waste valorization and biotechnological process was successfully provided.

Furthermore, a 4-question questionnaire was used to find out the students' opinion regarding the laboratory activity, with answers from 1 to 5, where 1 is "not at all agree" and 5 is "extremely agree" (see the SI). It is indicated that a high percentage of students (90%) strongly or somewhat agree that the active learning methodology proposed favored student motivation. 85% of students believe that the specific and transversal skills acquired in this academic activity are relevant for their future professions. Furthermore, over 70% of the students gave the highest rating for the laboratory proposal.

HAZARDS

Laboratory work must be carried out with a lab coat, protective gloves, and safety glasses. The management of NaOH and HCl solutions, corrosive compounds that may cause burns and skin irritation, is carried out in the fume hood. The enzymes used in the dissolution should not cause problems. The potassium sodium tartrate is not hazardous; 3,5-DNS and citrate buffer may cause skin irritation. Salicylic acid derivatives also may cause eye damage. Ethanol is flammable. Students must be careful when handling the centrifuge, the autoclave, and the hot water bath and follow the teacher's instructions. The laboratory is prepared with different bins for the disposal of the wastes generated.

https://doi.org/10.1021/acs.jchemed.4c00729
J. Chem. Educ. 2024, 101, 4405−4410

CONCLUSIONS

The students reinforced basic chemistry concepts such as the preparation of solutions, stoichiometry, etc. as well as knowledge about chemical and biotechnological processes applied to food waste upgrading. Moreover, the students became familiar with interesting analytical and mathematical tools.

The great work carried out by the students allowed us to largely achieve the stated objectives. The good results obtained demonstrated the great potential of the apple pomace as carbon and nutrient sources in second generation biorefinery processes. The proposed models allowed us to achieve a high degree of fit between the experimental and calculated data.

Students raised awareness of the need for replacing fossil fuels by second generation bioethanol, thus reducing crude oil dependence and the emissions of greenhouse gases and helping in mitigating the heating of Earth and climate change.

In short, the understanding of the students was improved with the implementation of this project, particularly in some points that are not adequately addressed during the course of their degree.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.4c00729.

Additional information for the students, competences, organization and experimental lab instructions, evaluation of work, students assessment, educational benefits and conclusions(PDF; DOCX)

Template for the laboratory report and guidelines for the oral presentation (PDF; DOCX)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Funding for open access charge: Universidade de Vigo/CRUE-CISUG.

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