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ORIGINAL ARTICLE

Virtual Laboratory Simulations in Biotechnology: A Systematic Review

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ABSTRACT

In biotechnology education, practical laboratory courses are vital. In having these practical courses, a lot of time and monetary resources are needed, it also requires the presence of the teacher and students and all the necessary equipment, which is currently not allowed in the Philippines due to the pandemic restrictions. Biotech virtual laboratries are useful in this time but what are the different research studies that has been done regarding this tool and what elements are important in terms of instructional design and technology? In this review, different types of technology and instructional design were examined, and overview of the previous research were highlighted in this paper. The study revealed that virtual laboratory simulations can be equally effective or better than hands on activity and said to be more effective compared to traditional teaching method. It was also found out that when the traditional teaching methods and virtual simulation laboratories yields better results in terms of performance. The included studies in this review utilized 2D, 3D, and VR technologies in having the virtual laboratory for the past few years. The review paper also identified different designs for instructions such as scaffolding and IBL (inquiry-based learnings). Biotech Virtual labs can be used as an effective alternative or complementary tool to an actual or physical conduct of laboratory experiment.

KEY WORDS: Virtual laboratories; biotechnology; online learning; systematic review

INTRODUCTION

Laboratory activities and work are often viewed as an essential part of science education. Chan et al. (2021) specified four essential skills that students should acquire during the conduct of laboratory experiments: (1) Skills related to science education, (2) scientific skills, (3) practical skills, and (4) general skills. Seery (2020) expressed that laboratory experiments are way different from the rest of the curriculum as it is a complex environment in which the learners need to draw the different skills that were presented by Chan et al. (2021). In this time of pandemic where practical and physical conduct of laboratory experiments may not be feasible, many online digital tools are available for use and one of this are the virtual laboratory simulations.

Virtual Laboratory Simulations are alternatives to the face-to-face or actual conduct of practical abilities (Ramadhan and Irwanto, 2018) that can be done through online distance learning. The emergence of these virtual laboratories happened because we are now in a fast-changing world in terms of technology, knowledge advancement, and the onset of the COVID-19 pandemic (Sypsas and Kalles, 2018). According to Udin et al. (2020), virtual simulations have lots to offer compared to the actual conduct of experiments such as greater accessibility, low cost, time saving, safe environments to chemical hazards and other dangers, flexibility, and self-paced learning. On the other hand, the advantages that the virtual laboratory can give depends on how the simulation is being used, learning without classmates and physical teacher, and the feel of a real laboratory are some of the drawbacks of virtual laboratories (Chan et al., 2021).

Studies and related studies regarding virtual laboratories are not new topics. In fact, many reviews had been published that compared virtual simulations and distance/remote laboratory classes to the traditional hands-on minds on laboratories. Most of these reviews are focused on lab practices across disciplines (Earth science, biology, chemistry, and physics) but very few reviews have focused on the use of virtual labs in biotechnology.

Sypsas and Kalles (2018) were able to be analyzed 29 research journals that underwent peer review which focused on virtual laboratory simulations in the discipline of biology, biotechnology, and chemistry. The reviewed articles focused on its effectiveness and the educational approaches as a supplementary tool for instruction. This study implied that virtual simulations show most likely the same to better results than the traditional way of learning laboratory activities for secondary (high school) education. In the review done, the commonly used approaches were blended and inquiry learning.

Ali and Ullah (2020) and Bellou et al. (2018) conducted a literature review on digital learning technologies in primary and secondary chemistry education. These two studies

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proposed and looked into the different pedagogical approaches, graphical interfaces utilized in the simulations, technologies in learning, learning outcomes of the study, and the research methods. Their findings for the studies suggested that the use of virtual laboratories has positive outcomes and however, some more efforts are to be done such as a meta-analysis of the studies. Ali and Ullah (2014) also emphasized the similarities and difference between 2D and 3D virtual science simulations and found out in their study that majority of the virtual simulations do not provide any guidance on how the experiment should be done.

This paper's systematic review is different from the reviews conducted by other researchers, because of the focus on virtual laboratory simulations that involve biotechnology concepts. Even though the study of Sypsas and Kalles (2018) mentioned biotechnology laboratory simulations, it was not that thorough nor was virtual reality any part of the review. Moreover, this study provides a holistic overview of the literature reviews of the different studies that involves biotechnology laboratory classes whether online or face-to-face learning that utilizes simulations.

The main goal of this systematic review is to provide an extensive review of the conducted research about virtual laboratories in biotechnology education and other related sciences. This study seeks to answer the following questions:

1. What are the main purposes, evaluation method, and learning outcomes of the research studies on using virtual simulations in biotechnology?
2. What are the different technologies being used for virtual biotechnology laboratory and its current trends?
3. What learning theories and instructional design features have been applied to biotechnology laboratory?

METHODOLOGY

In conducting this systematic review, the researchers utilized and followed PRISMA's guiding principle (Moher et al., 2009). This principle helped the researchers to conduct the research in a more complete and transparent manner. The process required the researchers to select appropriate criteria, selection of articles, searching strategies, and data collection process.

The first step done was to search for the literature in online data bases such as Google Scholar, Web of Science, Elsevier, Scopus, and PubMed. In looking for articles and journals, the researchers used different terms to look for publications. The following words or phrases were used: Biotechnology Virtual Laboratory or Biotech Virtual Lab or Simulations, Biotechnology Experiments and Simulations, Biotechnology simulations, or Biotech Virtual Simulation.

These search key words should be part of the title, abstract keywords, or abstract of a publication between 2005 and 2021. The search resulted in 580 studies all in all when added from the different databases, and these studies were then screened further (Table 1).

The next step that should be undertaken when doing a systematic review is selecting the relevant publication articles to be included in the review by searching the title and abstract of each record. After initial screening, 87 studies were found to be relevant and valid publications. The other 493 studies were removed. The selected publications were then further screened using their full text version. The researchers filtered out the publication further using the criteria did they contain a virtual environment representation. For the last screening process, the researchers looked for the type of interface being utilized by the simulation whether 2D, 3D, or virtual reality and these should be included in the text or images in the publication. After the thorough screening, 22 publication articles remained to be included in the review.

The last step that was done was the coding and data analysis. The 22 studies relevant information that were appropriate for the research questions were coded. The variables were then classified into categories through a spreadsheet.

FINDINGS

This part of the research connects the result of the review inquiry to the to the three research questions mentioned and is divided into different sections: research methodology, technology, and institutional design.

Research Purposes

There were three research categories in this study which the purpose of the publication is anchored, these categories were comparative, evaluative, and technical study. Based on the analysis of this study, the evaluative study had the greatest number of in the searched publications with 45.5%, followed by technical study with 32% and lastly, comparative with 22.5% of the relevant publications.

Evaluation Method

In evaluating the effectiveness of virtual simulations, different research methods were utilized such as qualitative, qualitative,

Table 1: A list of inclusion and exclusion criteria used to select relevant articles from the database

Inclusion criteria	Exclusion criteria
Journal and conference proceedings	Review, abstract, and non-peer reviewed publications
Virtual Laboratories used for Science, Biology and Biotech education	Publication with full text that is not accessible
Contains Biochemical Laboratory practices or laboratory safety	Virtual application that is only used to teach chemical concepts (e.g., Molecule visualization, periodic table)
Uses 2D, 3D, 4D, VR interfaces or immersive virtual reality devices	Virtual Lab application that requires the real environment (augmented reality)
The publication must be in English	Publications that are not in English

and mixed method that was used in measuring different domains or skill-based learning outcomes. There are notable differences in the use of these methods between evaluative and comparative studies.

In the reviewed publication the widely used evaluation method for comparative studies was the test, and on the evaluative studies, questionnaires were the widely used material. In addition, qualitative evaluation was used to measure the affective outcomes of the participants in the comparative studies. In contrary, evaluative studies utilized more real-time assessments compared to comparative studies.

Learning Outcomes

In the investigation of the learning outcomes, the researcher has observed that majority of the comparative studies measured cognitive learning outcomes, while the evaluative studies measure the affective outcomes. Despite the prior findings, participants' affective outcomes were still evaluated in comparative studies. This is because the studies were able to evaluate the usability of the virtual laboratory and opinions of the participants based on the comparative analysis.

Technology

In this study, technology was reviewed in two categories: the technological trends and display technology and interface. In the reviewed publication, display technology in the biotechnology virtual simulations refers to the visual display of the biological instruments and reagents in a virtual classroom environment. The commonly used interface in these types of technologies was three-dimensional displays (n = 15, 68%) and others used 2D desktop display (n = 5, 23%) and virtual reality (n = 2, 9%) some used HMD devices, Oculus Go and Rift, and Samsung gear. It was also found out from the review that since 2000 until today 2D and 3D technologies are the most prominent display technologies throughout these years, even though the virtual reality has slowly emerged from 2012 until present. It should be noted that virtual reality technologies were widely used in the year 2018 as when internet technology has become more available in developing countries.

Instructional Design

Under the instructional were two different areas that were reviewed such as learning theories and instructional support. It was found that learning theories were manifested in the reviewed publications, they were indicated in the keywords, and on the shortened title of the publications. The theories that were included in the review were discovery learning (n = 3), independent learning/self-paced learning (n = 4), inquiry-based learning (n = 5), and learning by doing (n = 3). These learning theories were the commonly used for biotechnology virtual laboratory. Some of the reviewed studies did not specify the type of learning theories that they have used in their study.

The second category under instructional design was the instructional support. During the review the following instructional support elements have emerged: Scaffolding or guidance, feedbacking, personalization, modality, and reflection. Similar with the instructional support, some of the reviewed articles did not indicated any of the instructional support. In the study of Ali and Ullah (2020), scaffolding was said to contribute better to the procedural understanding of the students compared to the no guidance learning. In a similar way, Borek et al. (2009) studied the effect of guidance to students in a form of tutors, or direct instruction. They found out that scaffolding resulted in better conceptual and procedural understanding compared to inquiry-based and directed instructions. In both studies, they have recommended that learners needed sufficient guidance in using the virtual laboratory environment/simulations.

DISCUSSION

Biotechnology Virtual Laboratory Research

In this systematic review, it was found out that most of the published journal articles performed comparison of the different media which led to comparing the virtual laboratory simulations with the traditional teaching styles. Quantitative methods were utilized in comparing the different the different approaches in laboratory teaching some of the forms of evaluation were knowledge test for cognitive competence and practical assessments such as actual laboratory performance to assess laboratory practical skills. In this review paper, declarative knowledge was the mostly studied leaning outcome which is also similar to other studies (Brinson, 2018). Moreover, qualitative methods of evaluation were also conducted using interviews, observations, and questionnaires to conduct a comparative study.

The comparison studies found out that the effectiveness of the virtual simulation or the virtual laboratory vary depending on the type of traditional method, they are compared with. In comparison to the passive media such as traditional lectures, radio lessons, and video or text presentation, virtual laboratory was found to be more effective for learning the basic facts about biotechnology and other allied courses. With this, it is imperative that the virtual simulations can match to the passive media. The study of Makransky et al. (2019) support this finding, it was elaborated in their paper that overloading of cognitive capacity using the virtual reality shows a better result when learners need to reason and apply biotechnology concepts in solving problems (Jagodzinski and Wolski, 2014; Makransky et al., 2019). In the conduct of laboratory classes, virtual laboratories are effective ways in showing the students the microscopic domains of biosystems, chemical compounds and more importantly they are manipulative and interactive (Herga et al., 2015). In the study of Tatli et al. (2010), the researcher found out that combinations of media visual support and inactivity.

In comparing the virtual laboratories with the traditional conduct of experiments, it was found out that the virtual laboratory simulations (VLS) are almost or equally the same or sometimes better than the hands-on laboratory activity in terms of effectivity as regards to the skill-based knowledge,

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declarative knowledge, and procedural understanding. Brinson (2015) and Sypsas and Kalles (2018) support the claim that the virtual laboratory is equally effective or better than traditional laboratories. On the other hand, it was argued that virtual laboratories cannot replace the hands-on activities, and a very limited evidence was found that VLS was worse than that of hands-on labs (Faulconer and Gruss, 2018). This implies that students have learned procedural knowledge and laboratory skills in virtual platforms even physical interaction was very limited or none (Pyatt and Sims, 2012). Moreover, Ullah et al. (2016) emphasized that when procedural guidance was provided during virtual experiments learners will perform better compared to student who attended the physical hands-on experiments. To justify this claim and strengthen this, more research should be done because there are limited studies regarding the difference in effectiveness of the two modalities. Kolil et al. (2020) said one factor that should be look into is the complexity or the simplicity of the experiments in virtual laboratories that will enable students to master or acquire skills and laboratory techniques.

The second research purpose of this review is to investigate the evaluative studies about virtual labs. The consideration that was employed in this study was the following: The class should be using biotechnology laboratories to evaluate the affective domain learning outcomes of the learners with questionnaires as the most utilized evaluation method, followed by interview and observations. Chan et al. (2021) said in their paper that positive attitude toward biotechnology, better usability of virtual simulation and considerable perceived self-efficacy are some of the users' revelation about the effects of VLS. In general, VLS is satisfying, helpful for grasping the lessons, and requires lesser amount of time than the traditional hands-on laboratory.

Technology used in Virtual Biotechnology Laboratory

In this review study, the different technologies being used were the 3D, 2D, and virtual reality. In the study of Ali and Ullah (2020), same technologies were reviewed; however, in this study, the researcher included the virtual reality which is an innovative technology that is not the same as two- and three-dimensional displays on desktops, and through this virtual laboratory simulations become realistic and interactive.

The virtual biotechnological laboratory simulations with two-dimensional display (2D) have been used to allow students to experience the simple visualizations and simulations of the experiments. The interface of a 2D display is easier to navigate, comprehensible animations which help improve the understanding of the learners regarding molecular to macroscopic applications of biotechnology in experiments. This interaction that is happening between students and the simulation offers a big advantage to the students who's learning biotechnology compared to those who are using the traditional media. Furthermore, using virtual simulations, the experiments become free and readily available always because it does not require a laboratory environment. Despite the benefits and advantages that the VLS offer, there are some drawbacks that emerges such as providing realistic laboratory experience to students to acquire necessary laboratory skills (Ali and Ullah, 2020; Qvist et al., 2015).

Most of the articles that has been reviewed in this study used three-dimensional technology for the VLS. This were developed to make the virtual laboratory more realistic and more accurate representation of the equipment and laboratory environment as well as the manipulation of apparatus are far better than the two-dimensional technology. Qvist et al. (2015), Winkelmann et al. (2014) also mentioned in their papers that VLS that used 3D technology enabled students to explore, and freely manipulate the interface with this the authors agreed that the level of interactivity and the realistic effect of the VLS can help students to be familiar with the concept of the laboratory before going to the actual real laboratory. The study Makransky et al. (2019) emphasized that another salient feature of a realistic virtual laboratory is the simulation of hazardous events that were dangerous when experience in real life. Some of these are the unsafety handling of reagents, breaking of some glass wares, and even the good practices in the can be simulated with this learning continues without sacrificing the safety of the children, and the most important thing is they learn. In this 21st century, technology has evolved and developed drastically, with this computer technologies improvement was made easier and better to realize the simulations into a new level of realism and interactivity which is very far during the time that the computer age is starting. Despite the advancement that the 3D simulations have achieved, it is still projected in the computers and still limited. It cannot yet give that same feeling of having the practical and hands-on laboratory class in reality (Winkelmann et al., 2014.)

Recently, virtual reality technology has emerged in various industries such as entertainment, education, medical, and many more. In the education industry, the virtual laboratory has a promising application as a tool for science subjects specifically to biotechnology. With this high-definition media virtual reality devices, the feeling of being in the real scenario in a virtual environment which is far different from the low immersion technology such as 3D and 2D technologies (Buttussi and Chittaro, 2018). Despite the promising technology of VR, it was reported that in terms of acquisition of conceptual knowledge, it is equally effective as 2D and 3D passive and physical laboratory (Dunnagan et al., 2019; Makransky et al., 2019). VR has many drawbacks too just like the 2D and 3D technology such as expensive, social isolation, and can cause simulator sickness/dizziness (Chan et al., 2021).

Instructional Design of Virtual Biotech Laboratory

In this systematic review, the researcher has investigated which theories, instructional support elements were implemented in the biotech virtual laboratories. Hew et al. (2019) stated in their paper that inquiry-based learning, discovery learning, learning by doing, and experiential learning were the mostly used theories because as time goes by these theories are evolving

and becoming more applicable and relevant when conducting a laboratory class. The autonomous and interactivity learning is aspects of the biotechnology virtual laboratory that enable learning environments to be constructivist and learner centered. This allows the learner to create more meaningful understanding of biotech concepts.

Another aspect of instructional design is the instructional support that learners enjoy during the virtual learning simulation experience (Chan et al., 2021). In the study of Makransky et al. (2019), the effective learning in virtual environment was hindered by cognitive overload or over information of the learners. This suggests that providing instructional support could manage this cognitive load better and could assist learners when needed. Modality, guidance, and feedbacking are some of the instructional support elements that were used in most of the review articles. However, in the reviewed articles, these instructional supports were mentioned very briefly and some of the topics can be learned more when reviewed studies have performed value-added research on principles of instructional support. According to the studies of Borek et al. (2009), Georgiou and Pinayotis (2007), and Jagodzinski and Wolski (2014) that virtual laboratories and simulations were most effective when support in terms of instruction and materials were given in learning content of biotechnology using audio media and with proper scaffolding. Because of the limited number of studies that have been conducted about biotechnology virtual laboratory, the researcher has come to the conclusion that focus more on how virtual laboratory on biotech were designed is needed than comparing the instructional media. Through this, we can find a more meaningful, more effective, and organized way in understanding the virtual laboratory system.

CONCLUSION

The conducted systematic review has been done in biotechnology virtual laboratories. This reviewed focused not only on the utilization and effectiveness of virtual laboratories but also to the included a thorough analysis of technologies and instructional design being used in doing the laboratory activities.

Based on the review, it can be concluded that virtual biotech laboratories can be used as an effective complementary or support tool or even an alternative to the physical hands-on laboratory activities, despite the argumentation presented by some authors that it can never be used as a replacement. In considering learning outcomes of all domains such as cognitive, affective, and skill-based or psychomotor, virtual laboratories can provide better outcomes and it can be claimed that virtual laboratories are equally effective as the traditional media or sometime better that it. The literature has suggested also that virtual laboratories are more effective when combined with the hands-on laboratory activities, but to achieve this utmost consideration should be given to the choice of technology and instructional design.

Technologies used in biotechnology virtual laboratories were from simple to sophisticated one such as two-dimensional (2D) graphics, to more sophisticated three-dimensional (3D) graphic representation of an actual laboratory. Despite the popularity and the utilization of the 3D compared to 2D and Virtual reality, each of these technologies have their own advantages and disadvantages, and they have different purposes. Some of the characteristics of these are the low cost, easy to manipulate which 2D can offer. On the other hand, 3D can give some interactions with that of an actual laboratories and can replicate experiments. Finally, the virtual reality can offer much of the realistic view of the laboratory and can give the learner a more profound experience of the actual laboratory.

The researchers also found that most of the studies included in the review did not consider instructional support or learning theories in designing the instruction. On the other hand, studies suggest that offering of scaffolding and introduce the instructional support to them will help learners to have a better cognitive load.

Ethical Statement

Ethics was not required for this study because this is a systematic review, and it uses data from open access journals which are publicly accessible data.

REFERENCES

Ali, N., & Ullah, S. (2020). Review to analyze and compare virtual chemistry laboratories for their use in education. *Journal of Chemistry Education*, 97(10), 3563-3574.

Bellou, I., Papachristos, N.M., & Mikropoulos, T.A. (2018). Digital learning technologies in chemistry education: A review. In: Sampson, D., Ifenthaler, D., Spector, J.M., & Isias, P., (Eds.), *Digital Technologies: Sustainable Innovations for Improving Teaching and Learning*. New York: Springer International Publishing. pp. 57-80.

Borek, A., Mclaren, B., Karabinos, M., & Yaron, D. (2009). How much assistance is helpful to students in discovery learning? *Lecture Notes in Computer Science*, 5794, 391-404.

Buttussi, F., & Chittaro, L. (2018). Effects of different types of virtual reality display on presence and learning in a safety training scenario. *IEEE Transactions on Visualization and Computer Graphics*, 24(2), 1063-1076.

Chan, P., Van Gerven, T., Dubois, J.L., & Bernaerts, K. (2021). Virtual chemical laboratories: A systematic literature review of research, technologies and instructional design. *Computers and Education Open*, 2, 100053.

Dunnagan, C.L., Dannenberg, D.A., Cuales, M.P., Earnest, A.D., Gurnsey, R.M., & Gallardo-Williams, M.T. (2019). Production and evaluation of a realistic immersive virtual reality organic chemistry laboratory experience: Infrared spectroscopy. *Journal of Chemical Education*, 97(1), 258-262.

Faulconer, E.K., & Gruss, A.B. (2018). A review to weigh the pros and cons of online, remote, and distance science laboratory experiences. *The International Review of Research in Open and Distributed Learning*, 19(2), 155-168.

Georgiou, S., Stavrinides, P., & Kalavana, T. (2007). Is victor better than Victoria at maths? *Educational Psychology in Practice*, 23, 329-342.

Hew, K.F., Lan, M., Tang, Y., Jia, C., & Lo, C.K. (2019). Where is the "theory" within the field of educational technology research? *British Journal of Educational Technology*, 50, 956-971.

Herga, N.R., Glažar, S.A., & Dinevski, D. (2015). Dynamic visualization in the virtual laboratory enhances the fundamental understanding of chemical concepts. *Journal of Baltic Science Education*, 14(3), 351-365.

Jagodzinski, P., & Wolski, R. (2014). The examination of the impact on students' use of gestures while working in a virtual chemical laboratory for their cognitive abilities. *Problems in Education 21st Century*, 61, 46-57.

Kolil, V.K., Muthupalani, S., & Achuthan, K. (2020). Virtual experimental platforms in chemistry laboratory education and its impact on experimental self-efficacy. *International Journal of Educational Technology in Higher Education*, 17, 30.

Makransky, G., Borre-Gude, S., & Mayer, R.E. (2019). Motivational and cognitive benefits of training in immersive virtual reality based on multiple assessments. *Journal of Computer Assisted Learning*, 35(6), 691-707.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., & PRISMA Group. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Medicine*, 6(7), e1000097.

Pyatt, K., & Sims, R. (2012). Virtual and physical experimentation in inquiry-based science labs: Attitudes, performance and access. *Journal of Science Education and Technology*, 21, 133-147.

Qvist, P., Kangasniemi, T., Palomäki, S., Seppänen, J., Joensuu, P., Natri, O., & Nordström, K. (2015). Design of virtual learning environments: Learning analytics and identification of affordances and barriers. *International Journal of Engineering Pedagogy (iJEP)*, 5(4), 64-75.

Ramadhan, M.F., & Irwanto. (2017). *Using Virtual Labs To Enhance Students' Thinking Abilities, Skills, and Scientific Attitudes*. In: International Conference on Educational Research and Innovation (ICERI 2017). pp. 494-499.

Seery, M.K. (2020). Establishing the laboratory as the place to learn how to do chemistry. *Journal of Chemical Education*, 97(6), 1511-1514.

Sypsas, A., & Kalles, D. (2018). *Virtual Laboratories in Biology, Biotechnology and Chemistry Education: A Literature Review*. In: ACM International Conference Proceeding Series. pp. 70-75.

Tatli, A., Bridgstock, R., Lettice, F., & Ozbilgin, A. (2010). Diversity management for innovation in social enterprises in the UK. *Entrepreneurship and Regional Development*, 22, 557-574.

Udin, W.N., Ramli, M., & Muzzazinah. (2020). Virtual laboratory for enhancing students' understanding on abstract biology concepts and laboratory skills: A systematic review. *Journal of Physics Conference Series*, 1521(4), 042025.

Winkelmann, K., Scott, M., & Wong, D. (2014). A study of high school students' performance of a chemistry experiment within the virtual world of second life. *Journal of Chemical Education*, 91(9), 1432-1438.