MAPPING LITERATURE REVIEW ON FERMENTATION SUBSTRATES FOR PRODUCTION OF LACTIC ACID BY *RHIZOPUS* SP

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ABSTRACT: Lactic acid has been a great interest to many industries such as food and beverages, cosmetic, chemical and pharmaceutical due to its various functions. It can be produced by *Rhizopus sp* through fermentation from various substrates such as glucose, starch and lignocellulosic materials. An effective fermentation process depends on the types of substrates which consists of different chemical constituents. This will lead to different production yields. To our knowledge, classification of studies according to the substrates used in the fermentation medium has never been conducted. Thus, the main objective of this study is to classify the earlier works on lactic acid production by *Rhizopus sp* on the most studied substrates. This study also aims to identify the location or country that focuses on lactic acid production from raw starchy-materials and the production yield. In this study, a mapping literature study was conducted to classify and structure relevant empirical studies into the types of substrates used. Network visualization based on keywords co-occurrences from selected bibliographic databases is included in this study as well. Eleven (11) substrates were identified from 171 empirical studies. Thirty-two (32) studies were discovered to use starch as substrates in lactic acid production by *Rhizopus sp*. Out of 32 studies, only twelve (12) studies reported the utilization of raw starchy-materials in their studies where most of the studies are reported from the United states. This study redounds to the researchers to uncover critical areas in the production of lactic acid especially in the use of substrates for novel approaches thus, a new hypothesis can be made.

KEY WORDS: *Fermentation, Fungi, Lactic acid, Rhizopus, and Substrates.*

1. INTRODUCTION

Lactic acid has attracted many interests as it has multifunctional uses in various industries and have attracted attention of lucrative and conservationist to its wide applications. The demand of lactic acid, which is the precursor to produce polylactic acid (PLA) is kept increasing. This is due to the emergence of PLA as one of the alternatives to non-biodegradable plastic that has the potential to reduce the environmental issues such as effects on marine life and land, water pollution and others. Also, it is reported that the highest demand for lactic acid is in the food-related industries which is 85% while 15% is for non-food industrial purposes [1].

Lactic acid can be produced via chemical synthesis or fermentation process. Hydrolysis of lactonitrile is one of the methods used in the chemical synthesis to produce lactic acid. Lactonitrile is a product derived from acetaldehyde and hydrogen cyanide by going through petrochemical process. Chemical synthesis however, will lead to the formation of racemic mixture of two optical isomers which are D(−)-lactic acid and L(+)-lactic acid. Also, this method is unsustainable due to the depletion of natural resources in the future [2]. Other than that, lactic acid can be produced by fermentation of bacteria such as lactic acid bacteria (LAB) or fungi. Production of lactic acid by fermentation is the most preferred method due to its stereoselectivity to produce only optically pure lactic acid [3].

An effective fermentation process depends on the types of substrates used [4]. Generally, different substrates have different chemical constituents such as carbohydrates, proteins and fatty acids. These varying chemical compositions of raw starchy materials are the factor that affects lactic acid yield and purification process. When simple sugar is used, it results in a lower purification cost but high raw material price. There are several substrates that have been studied and used in the fermentation process for the production of lactic acid which are glucose, starchy materials, lignocellulose biomass, whey, food waste and microalgae [4].

Rhizopus sp is one of a specific genus of molds which is known as spoilage organisms. *Rhizopus sp* is considered as one of important organisms in producing fermented food like tempeh and ragi and chemical as well as biotechnology products [5]. In lactic acid production, *Rhizopus sp* has been used by many manufacturers worldwide because of its validity as generally regarded as safe (GRAS) organisms. Besides, it is capable to utilize cellulose, starch as well as pentose sugar obtained from agricultural remnants which are relatively cheap raw materials due to the release of hydrolytic enzymes [6].

Mapping literature study which is also sometimes referred as scoping study is a method to conduct research by means of classification and counting contributions in the context of the categories of that classification. Also, this research method is usually conducted to provide a broad review of the interested topic area. Besides, this type of research method requires searching the literature on bibliographic databases or journal publications to identify the contribution of the literature as well as the location of published literature [7]. This study employs three bibliographic databases which are PubMed, Scopus and Dimensions.

This study is intended to perform a mapping literature study on the production of lactic acid by *Rhizopus sp* in which the focus is on the types of substrate used in the fermentation medium. Besides, this study also visualizes the network that is based on keywords co-occurrences among the studies according to each bibliographic database.

2. METHODOLOGY

2.1. Research Question

This mapping study was based on the identification of all related and relevant empirical studies of lactic acid production by *Rhizopus sp*. The aims are to classify the substrates used in the empirical studies according to their types and to identify research gaps. This formulation strategy leads to the following research questions (RQs):

Primary RQ: What kind of substrates were used to produce lactic acid by *Rhizopus sp*?

Sub-RQ1: Which country focuses on raw starchy materials as the substrate to produce lactic acid by *Rhizopus sp.*?

Sub-RQ2: What is the production yield of the lactic acid identified from Sub-RQ1?

2.2. Sources of Evidence

In this study, three online databases were used to find the related articles which are PubMed, Scopus, and Dimensions. PubMed, Scopus, Web of Science and Dimensions are supported bibliographic databases in VOSviewer. Web of Science was excluded due to access limitations.

2.3. Search Strategy

The search strategy of this study was based on the title and abstract specifically from empirical studies articles and must be related to the production of lactic acid by *Rhizopus sp*. Thus, the search string strategy that was used by Zein et al.[8], was implemented in this study. The first strategy was to apply synonyms of the keywords used. In this study, lactic acid and *Rhizopus sp* were the keywords applied to search the related articles. Lactic acid is a chemical substance therefore, it has several other names that are often used such as lactate, 2-hydroxypropanoic acid/ 2 hydroxypropanoic acid and 2-hydroxypropionic acid/ 2 hydroxypropionic acid. As for *Rhizopus sp*, no similar keyword was used since it is already a general keyword. To incorporate the different spellings and synonyms of lactic acid, Boolean OR was used in the search string. Also, since there are two keywords used in the search string, thus Boolean AND was used to connect both important terms together.

2.4. Selection Criteria

The selection criteria consists of two conditions which were inclusion and exclusion. Generally, this process was done to screen and remove studies that are irrelevant to the proposed RQs as well as the purposes of the study. Following inclusion criteria were employed in this mapping study:

- Empirical data and supporting evidence must be included in the studies.

Exclusion criteria applied were:

- Expressed enzyme of *Rhizopus sp* into another microorganism,
- Lactic acid that is produced in the muscles,
- Opinion-based studies without any supporting evidence,
- Papers not written in English.

2.5. Keywording of Abstracts (classification scheme)

There are two processes of keywording that were suggested by Petersen et al., [9]. First, the context of the studies was determined by identifying the keywords as well as the ideas of the articles that reflect the nature of the studies. Then, the same keywords from different studies were collected and combined with the purpose of obtaining an exact understanding of the research. By doing so, a set of categories under certain population can be identified. However, there might be some articles that show low standard of abstract which make it difficult to identify the keywords. In this case, reviewing the introduction as well as the conclusion of the papers can help in searching for the right keywords. The first phase of this study was only focused on the title and abstract of the studies (i.e., lactic acid production by *Rhizopus sp*). Then, during the second phase, a set of clusters were formed based on the types of substrates used such as glucose, starch, lignocellulosic materials, food waste, glycerol and microalgae by reading the introduction, methods and the conclusion of the studies as suggested by Zein et al. [8].

2.6. Data Extraction and Mapping of Studies

The bibliographic data were downloaded from each online database. The format used to download the data from PubMed, Scopus and Dimensions were text (txt), Research Information System (RIS) and Comma Separated Value (CSV) respectively. The database was then entered in the VOSviewer to extract keywords from titles and abstracts by using text mining function in the software. This function can analyze the co-occurrence of keywords from the database and present it on a two-dimensional map [10]. The map was generated by setting the software: binary counting, disregarding repetitions, and keywords with at least five co-occurrences. Filter feature was also used to filter irrelevant terms such as articles and prepositions. Manual filtering was also applied to exclude unrelated expression after the filtering process was done by the software [11].

3. RESULTS AND DISCUSSION

3.1. Search Results

Table 1 shows the preliminary search results as well as two phases of filtered studies for each online database. A total of 786 articles was returned from all sources which then reduced after filtration steps were applied as discussed in Section 2.6. As for Phase 1, after filtering the studies by going through the title and abstract, a total of 371 studies remained. After considering the inclusion and exclusion criteria for the second phase, 74 studies/articles were removed. Most of the remaining studies in Phase 2 are from Scopus, Dimensions and PubMed which each at 161 (54.21%), 70 (23.57%) and 66 (22.22%) studies, respectively. However, all 66 works from PubMed were found in Scopus whereas 22 out of 66 works of PubMed were found in Dimensions. About 41 works from Dimensions are identified in Scopus as well but none of Scopus and Dimensions works is discovered in PubMed, which make the net total of studies is 171. The number of publications for all studies regarding lactic acid production by *Rhizopus sp* between the years 1989 and 2021 is shown in Fig. 1.

Table 1: The remaining studies after each filtration step

Online database	Search results	Phase 1	Phase 2
PubMed	173	70	66
Scopus	477	223	161
Dimensions	136	78	70
Total	**786**	**371**	**297**

Fig. 1 depicts fluctuating number of publications throughout 1989 to 2021. Between the years 1989 and 2002, this area of study was still underdeveloped where the number of publications for each year was not more than six publications. Also, there is no publications regarding lactic acid production by *Rhizopus sp* for two consecutive years which are 2000 and 2001. As the year increased, the number of publications started to increase in fluctuating trend where the highest number of publications was in 2009 with 16 publications. However, the interest in this area started to drop again from 2017 onwards.

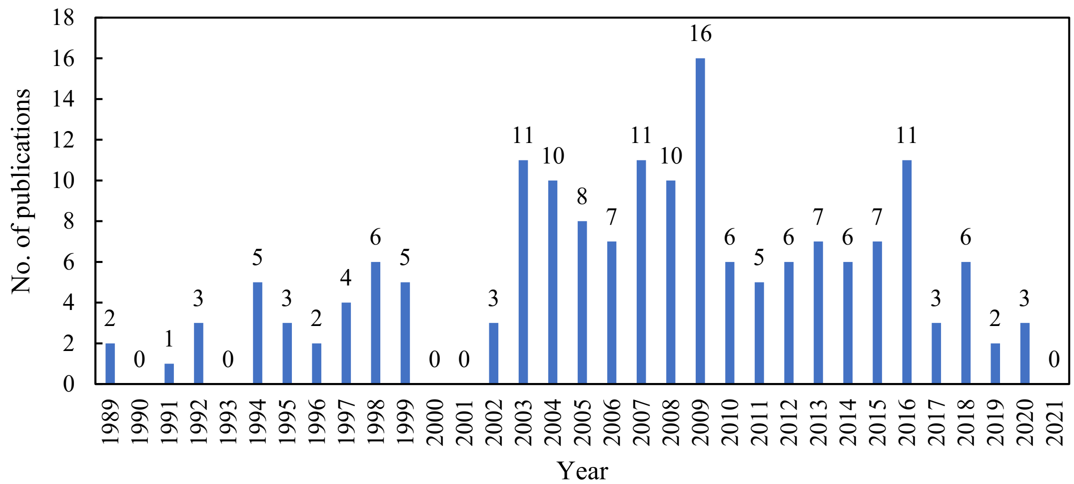

Fig. 1. The number of publications regarding lactic acid production by *Rhizopus sp* per year.

3.2. Co-occurrences Network of Term (keywords)

Keyword co-occurrence is a type of analysis that extract repetitive keywords from the content to identify the cognitive structure and important topics of the academic field studied [12]. The network visualizations for each bibliographic database (*i.e.*, PubMed, Scopus and Dimensions) are presented in Fig. 2 to 4. After filtering unnecessary keywords in VOSviewer software, only two clusters are identified for each bibliographic database which can be distinguished by colors of green (Cluster 1) and red (Cluster 2). The formation of cluster is based on keywords that have strong connection with a keyword of highest frequency. The main keyword usually will guide the cluster. The number of clusters formed is dependent on the number of main keywords identified [13]. These clusters indicate the relatedness of publications in word relations, shared words in the titles, abstracts, or full texts of publication [14]. The cluster covered research topic of lactic acid production by *Rhizopus sp.*, which in this study is topic identification. Various research topics were identified by VOSviewer however, the topic is narrowed to substrates only. The clusters contain information of research topics that can help the researchers to focus on a specific research area.

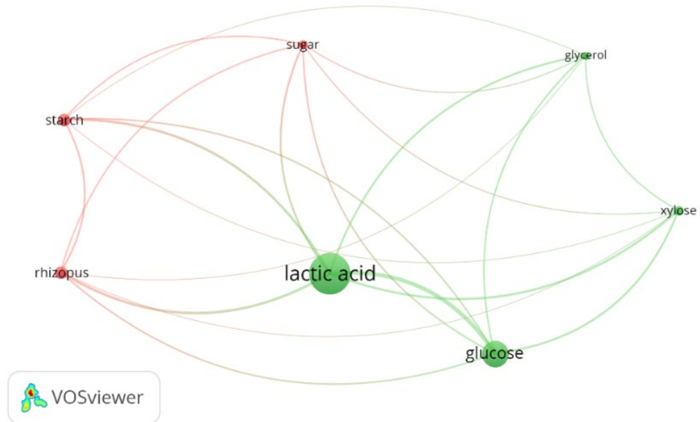

Fig. 2. Keywords and cluster by color for the search of "Lactic Acid" and "*Rhizopus*" from PubMed.

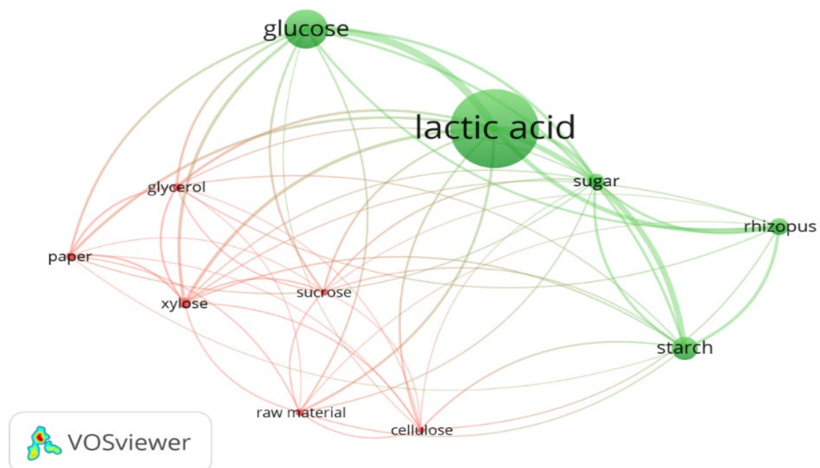

Fig. 3. Keywords and cluster by color for the search of "Lactic Acid" and "Rhizopus" from Scopus.

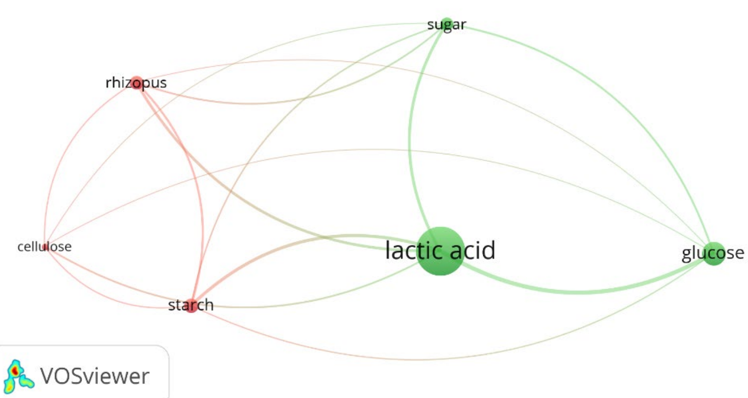

Fig. 4. Keywords and cluster by color for the search of "Lactic Acid" and "Rhizopus" from Dimensions.

In Fig. 2, Cluster 1 that is guided by keyword lactic acid is larger compared to Cluster 2, where the main keyword is *Rhizopus*. In Fig. 3, the size of Cluster 1 with the keyword of lactic acid is also greater than Cluster 2 in which xylose is the main keyword in cluster 2. Lastly, Cluster 1 where the dominant keyword is lactic acid can be observed to have bigger radius than Cluster 2 which is directed by *Rhizopus*. In addition, comparing Fig. 2 and 4, Fig. 3 visualizes more keywords in the map. This is due to the wide range of fields offered by Scopus database which resulted in numerous studies found [15]. Hence, more keywords can be observed in Fig. 3. In each figure, glucose keyword is observed to have the largest size among other substrates, and it is the most prominent in Fig. 3. This can be explained by the larger number of studies obtained from Scopus database (161 papers) compared to PubMed database (66 papers) and Dimensions database (70 papers). In addition, it shows the closest distance to lactic acid only in Fig. 2 and Fig. 3. The distance between two terms usually points out the fact of the terms being related with regard to co-occurrences. This means that the terms have a stronger connection and relatedness when the distance between the two terms are located close to each other [16]. Besides, it also can be observed that glucose link has the thickest lines with lactic acid and followed by starch in each figure. This line also indicates the frequency of co-occurrences between the two items which is also referred as the link strength [17]. This means that keyword glucose and lactic acid always occur together as glucose is the most frequent substrates that were used to produce lactic acid from *Rhizopus sp.*

Table 2 shows the analysis of keywords similarities among the three bibliographic databases. The reason of keywords similarities intersect among the three databases is due to the higher frequency of keyword co-occurrences extracted from each bibliographic database. Several keywords from PubMed and Dimensions are non-reticulate because of less co-occurrences identified or not exist at all. Thus, no similarities are found between the keywords.

Table 2: The similarities of keywords among the three bibliographic databases

Similarities of keywords	PubMed	Scopus	Dimensions
Glucose	X	X	X
Starch	X	X	X
Xylose	X	X	Not exist
Glycerol	X	X	Not exist
Paper	Not exist	X	Not exist
Raw material	Not exist	X	Not exist
Cellulose	Not exist	X	X
Sugar	X	X	X
Sucrose	Not exist	X	Not exist

3.3. Answering Research Question

Primary RQ: What kind of substrates were used to produce lactic acid by *Rhizopus sp*?

There are 11 types of substrates that were identified from all studies: glucose, starch, xylose (wood sugar), lignocellulosic materials, glycerol, different/other carbohydrates, microalgae, seaweed, gluconic acid, food waste and mixed substrate. These substrates were grouped by investigating the introduction, methods and conclusion of the studies as described in Section

2.6. Fig. 5 shows the classification of studies according to the substrates used in the production of lactic acid by *Rhizopus*. The classification of studies according to substrates used are presented in Table 3. The detail for each study is available in Table A.1, Appendix A.

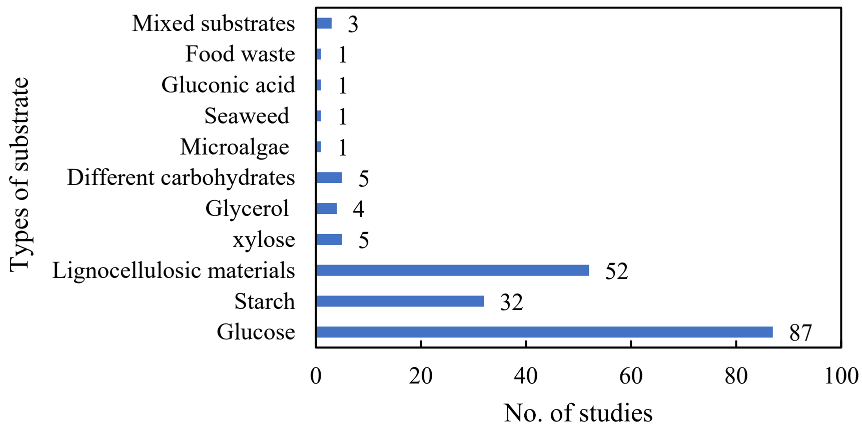

Fig. 5. Number of studies classified into types of substrates.

Table 3: Classification of studies according to substrates used

Types of substrates	Study	Total study
Glucose	S2, S8, S10, S15, S17, S19, S22, S23, S26, S27, S28, S29, S31, S34, S36, S38, S42, S44, S46, S51, S53, S56, S57, S59, S60, S61, S66, S67, S68, S70, S72, S73, S74, S77, S78, S79, S80, S81, S86, S87, S89, S91, S93, S94, S95, S96, S97, S98, S99, S102, S107, S108, S109, S110, S111, S119, S120, S125, S127, S128, S130, S131, S132, S133, S134, S135, S136, S137, S139, S140, S141, S142, S143, S144, S145, S146, S147, S148, S149, S151, S152, S155, S156, S157, S162, S168, S171	87
Starch	S4, S13, S14, S24, S37, S52, S54, S58, S63, S64, S74, S88, S90, S91, S102, S103, S104, S105, S111, S112, S116, S121, S122, S123, S127, S138, S142, S153, S158, S159, S161, S166	32
Lignocellulosic materials	S1, S3, S5, S6, S9, S11, S12, S15, S16, S18, S20, S21, S25, S30, S32, S33, S35, S40, S43, S45, S47, S48, S49, S50, S51, S63, S65, S69, S71, S72, S76, S82, S83, S85, S92, S100, S101, S102, S106, S108, S113, S114, S15, S117, S118, S124, S126, S129, S163, S167, S169, S170	52
Xylose	S51, S57, S66, S78, S84	5
Glycerol	S7, S28, S39, S62	4
Other carbohydrates	S16, S62, S75, S150, S160	5
Microalgae	S6	1
Seaweeds	S6	1
Gluconic acid	S27	1
Food waste	S41	1
Mixed substrates	S21, S51, S55	3

Substrates play important role in the production of lactic acid due to different chemical compositions of the substrate and their concentration in the fermentation medium. Futhermore, it influences the production cost of lactic acid. Pure substrate such as glucose and xylose will result in high yield of lactic acid with less chemical component to be purified. Hence, reducing the cost of purification. However, the raw material cost is one major drawback of the production. Exploring new substrate is the focus of many research. Still, there are many other factors influencing the cost and yield of lactic acid. Lignocellulosic materials for example, contain mostly cellulose, xylan, arabinan, galactan and lignin [18]. Pre-treatment of lignocellulose is required prior to the fermentation process. However, the presence of several compounds such as acetic acid, furfural and 5-hydroxymethylfurfural during the pre-treatment can act as an inhibitor in the fermentation process thus affecting lactic acid production [18]. Starch on the other hand, can be assimilated directly by *Rhizopus sp.*. Nevertheless, glucose is still the most preferred substrate. Glucose can be attained from starch materials by acid hydrolysis. The concentration of substrates in the fermentation medium also greatly affects lactic acid production. A high concentration of substrates could enhance the production of lactic acid, but too high concentration will cause the substrate to act as an inhibitor thus, reducing the production. Substrate concentration that is too low will also result in low production of lactic acid. Therefore, it is important to determine the optimum concentration of fermentation substrate to obtain a high lactic acid production. However, it is also crucial to note that different parameters and conditions of the fermentation process will also influence lactic acid production.

3.3.1. Glucose

According to Taskin et al. [19](S43) and Vially et al. [20](S62) glucose is considered as the most preferred carbon source for lactic acid production by *Rhizopus sp*. This explains the reason for numerous studies using glucose as substrate. A recent study presented by Yin et al. [21](S2) addresses the effect of *R. oryzae* LA-UN-1 morphology on L-lactic acid production by using glucose as the sole carbon source in the production medium. However, the focus of the study is to determine different protein expressions of cells grown in distinct conditions by using proteomics method. It concludes that pellet characteristic of *R. oryzae* LA-UN-1 is more preferrable in lactic acid production where it yields the highest concentration of lactic acid (approximately 62 g/L). Another study that focuses on the morphology especially pellet form of *Rhizopus sp.* for lactic acid production from glucose was reported by Fu et al. [22](S29).

An alternative fermentation process which is *in situ* extractive fermentation was studied by Matsumoto and Furuta [23](S8). The lactic acid was produced by *R. oryzae* IAM 6022 and glucose was used as the substrate. Compared to the conventional fermentation, *in situ* extractive fermentation could prevent low productivity of lactic acid as the accumulation of lactic acid in the broth is removed. Extractive fermentation was conducted in two different bioreactors which are stirred tank reactor under aerobic conditions and air-lift reactor. The study also compared the concentration of lactic acid production in aerobic and anaerobic conditions.

Immobilization techniques were also studied to improve lactic acid production using glucose as the substrate. Coban & Demirci [24](S23) investigated lactic acid production from glucose by immobilizing *R. oryzae* NRRL 395 in talcum and aluminum oxide microparticles so as to achieve smaller pellet size of cells. Wang et al. [25](S42) improve the productivity of L(+)-lactic acid by using modified polyvinyl alcohol (PVA) gel as a matrix for *R. oryzae* RQ4015 immobilization. *R. oryzae* RQ4015 was immobilized in alginate-modified and phosphate-modified PVA and fermented in a medium containing glucose. Additionally, a comparison of free and immobilization of *Rhizopus sp.* in PVA cryogel was also performed by Maslova et al. [26](S19).

3.3.2. Starch

The utilization of excessive glucose concentration for lactic acid production by *Rhizopus sp.* results in the limitation of the efficiency of the process [27]. Thus, many researchers replace glucose with other options such as starch. Most of the studies used potato or corn starch as the substrates and some conducted starch hydrolysis to get the fermentable sugars.

An optimization study was reported by Liu et al. [28](S105) where cull potatoes were used as the substrate. Three strains of *Rhizopus sp.* which are *R. oryzae* NRRL 395, *R. oryzae* NRRL 1472 and *R. oryzae* NRRL 1526 were screened for the ability to assimilate cull potatoes. Besides, utilization of both edible and inedible cassava was also investigated by Trakarnpaiboon et al. [29](S13) and Azmi et al. [30](S24), respectively. The former used cassava starch to screen thermotolerant *Rhizopus* strain that are capable of utilizing the starch for highest lactic acid production. It was then experimented for characterization of lactic acid production from cassava starch liquefied with α-amylase. Meanwhile, the later study employed two optimization processes in their study which are hydrolysis and fermentation. The hydrolysis process was to determine the optimum concentration of starch (starch concentration range: 1.5, 2.5 and 3.5 w/v%) to be used for the fermentation process. Then, the fermentation process was optimized by investigating the effect of different pHs, inoculum sizes, agitation rates and temperatures for determining the yield of lactic acid and ethanol.

A comparison between starch and glucose utilization by thermo-tolerant strains of *Rhizopus* which are TISTR 3514, TISTR 3518 and TISTR 3523 was presented by Kitpreechavanich et al. [31](S74). This study intended to evaluate organic acids produced by these strains and only total lactic acid which are L- and D-lactic acid and fumaric acid were measured. Research by Yin et al. [32](S142) also provides empirical data on using different carbohydrates such as glucose, mannose, fructose, sucrose, raffinose, inulin, maltose, rhamnose, xylose, galactose and corn starch where the highest lactic acid produced by *R. oryzae* NRRL 395 was from corn starch liquefied with α-amylase. Different concentrations of liquefied corn starch ranging from 100 to 240 g/L were then tested to determine the optimum concentration to be used in batch cultures afterwards.

3.3.3. Xylose

Saito et al. [33](S51) conducted an experiment to screen 31 strains of *R. oryzae* that produces lactic acid in a medium containing xylose. The selected strain was then used to study the kinetics of lactic acid production and substrates consumption (glucose, xylose and mixture of glucose and xylose). The fermentation of wheat straw was also reported in this paper by using the same strain of *R. oryzae*. Another study that contributes to the qualitative analysis of lactic acid production from xylose is from Guo et al. [34](S57). Prior to the fermentation of xylose, *R. oryzae* GY18 was tested for its tolerance in sucrose-containing medium with different concentrations (40–130 g). The fermentation was performed with different initial concentrations of xylose (20–100 g) and glucose (60–180 g). Conversion of corncob hydrolysate was also investigated in this study.

The influence of xylose on an original strain and mutated strain *of R. oryzae* PW352 was also analyzed by Wang et al. [35](S66). The mutated strain namely *R. oryzae* RQ4015 was also cultivated in a fermentation medium containing mixture of glucose and xylose where the initial concentration of xylose varied between 0 and 100 g/L resulted in 76 g/L of maximum lactic acid. Whilst Maas et al. [36](S78) studied xylose consumption by *R. oryzae* CBS112.07 by limiting the nutrient in the fermentation medium produced maximum of 24.4 g/L lactic acid. They also identified the enzymes involved in the xylose metabolism by the fungal cells. An optimization study of lactic acid production from xylose by *R. oryzae* RLC41-6, a mutant strain of *R. oryzae* PW352 was reported by Yang et al. [37](S84). The parameters involved in this experiment are initial xylose concentration (20–100 g/L), nitrogen sources, aeration, inoculum size, addition of $CaCO_3$ and concentration of ammonium sulphate.

3.3.4. Lignocellulosic Materials

After starch, lignocellulosic materials are regarded as the cheapest and extensively available to be used as carbon sources for lactic acid production [19]. Raw materials that can be considered as lignocellulosic materials are crop residues (i.e., bagasse, straw, stover, hulls, pulp), wood (i.e. hardwood, softwood), herbaceous biomass (i.e. any type of grasses), cellulose waste (i.e. recycled paper sludge, waste office paper) agro-industrial waste (i.e. kernels, pulp, sawdust, wood chips) and municipal solid wastes [38].

The most recent study that investigated the utilization of lignocellulosic material was conducted by Zain et al. [39](S1) where solid pineapple waste was used as the fermentation substrate. Preliminary experiment to determine the content of the solid pineapple waste was done prior to fermentation of lactic acid by *R. oryzae* NRRL 395. Material from herbaceous biomass which is *Sophora flavescens* was used to provide carbons source for *R. oryzae* 3.819 in a study performed by Ma et al. [40](S3). This material was tested in both separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) for lactic acid production.

There are two studies that reported the use of cellulose waste with regard to lactic acid production by *Rhizopus sp*. The first study is by Takano and Hoshino [41](S16) where the concentration of lactic acid produced by *Rhizopus sp.* was investigated from treated paper sludge at concentration of 50 and 100 g/L. This study also characterized lactic acid production from various carbohydrates and polyols which are glucose, galactose, mannose, fructose, xylose, arabinose, sucrose, maltose, cellobiose, lactose, glycerol, sorbitol, mannitol, soluble starch and α-cellulose. Next study is described by Dhandapani et al. [42](S5) where different concentrations of industrial wastepaper sludge (i.e., 50 g/L, 75 g/L and 100 g/L) were employed in their study together with the effect of temperature and pH in the fermentation process.

Various lignocellulosic materials were utilized in a study by Maslova et al. [43](S6) to produce various organic acids such as lactic acid, fumaric acid and succinic acid. The materials are wheat straw, rice straw, sugarcane bagasse, aspen sawdust, birch sawdust, pine sawdust, Jerusalem artichoke stalks and tubers. A study from Saito et al. [44](S165) explained the effect of extracted potato pulp at the intervals over 96 days on the composition of lactic acid produced in fermentation with or without *R. oryzae* NBRC 4707.

3.3.5. Glycerol

The idea of using glycerol as a substrate for fermentation of lactic acid has also gained significant interest from the researchers and there are several studies reported on it. According to Yuwa-amornpitak and Chookietwatana [45](S7), glycerol is one of incidental products formed during the manufacturing of biodiesel. In their study, waste cooking oil glycerol, a biodiesel by-product, was used as a substrate. The glycerol was mixed with cabbage extract for qualitative analysis of lactic acid production in batch by *R. microsporus* and was compared with cabbage extract without glycerol. The evaluation of lactic acid production from glycerol and glucose was carried out by Wang et al. [46](S28). The initial concentrations of both glycerol and glucose were varied from 10 to 70 g/L. This study explained the comparison of growth kinetics and extracellular metabolites, activity of lactate dehydrogenase and effect of temperature on glycerol consumption and synthesis of lactic acid under aerobic conditions. A comparative study between pure glycerol and crude glycerol was presented by Vodnar et al. [47](S39). Also, lactic acid production from crude glycerol supplemented with inorganic nutrients and with lucerne green juice was discussed in the study as well.

3.3.6. Other Carbohydrates

Other than glucose, various carbohydrates such as lactose, cellobiose, inulin, fructose etc. are extensively studied for production of lactic acid. In a research conducted by Vially et al. [20](S62), five carbohydrates (i.e., glucose, xylose, glycerol, sucrose and lactose) were used as carbon sources to determine the performance of lactic acid production by *R. oryzae* UMIP 4.77 in bioreactor at the fixed fermentation conditions. One of the earliest studies that investigated the effects of carbohydrates in the formation of lactic acid was by Rosenberg et al. [48](S150). Their study reported on the addition of various carbohydrates which are D-xylose, D-fructose, D-galactose, D-glucose, D-mannose, cellobiose, maltose, sucrose and starches to observe the formation of products. They also researched on various polyols as substrates for fermentation of lactate.

A relative study on lactic acid production by two different fungal cells which are *R. oryzae* NBRC 4785 and *Amylomyces rouxii* CBS 438.76 was written in detail by Watanabe and Oda [49](S75). Both fungal cells were tested for their tolerance on various carbohydrates such as glucose, sucrose, maltose, raffinose, fructooligosaccharides, inulin and soluble starch. In addition, they reported on the sucrose-hydrolyzing enzymes synthesized by both fungal cells and compared the analysis on the different purification steps of the sucrose-hydrolyzing enzymes. The synthesis of lactic acid and biomass using sucrose as the sole carbon source was reported by Lhomme and Roux [50](S154). Two methods of cultures strategy which are submerged cultures on thin liquid film and immobilization fibrous and particulate matrix were applied on *R. arrhizus* prior to the fermentation process. Utilization of carbon source and oxygen was measured and discussed too. Another investigation and analysis regarding the utilization of different carbohydrates were mentioned in Section 3.3.2 and 3.3.3, works from Yin et al. [32](S142) and Takano and Hoshino [41](S16), respectively.

3.3.7. Microalgae, Seaweeds, Gluconic Acid and Food Waste

As shown in Fig. 5, only one paper utilized microalgae, seaweeds, gluconic acid and food waste as the subtrate, respectively to produce lactic acid. These types of substrate have still not gained significant attention from many researchers which could be due to lack of information and studies reported related to these subjects. Maslova et al. [43](S6) investigated and discussed several types of microalgae (i.e., *C. vulgaris, Arthrospira platensis, Nannochloropsis sp, Nostoc sp* and *Dunaliella salina*) and seaweeds (i.e., *S. latissimi, Ulva lactuca, Asparagopsis taxiformis, Gracilaria tenuispititata* and *Gelidium amansii*) used for organic acids production. Shu et al. [51](S27) reported on the use of gluconic acid as a substrate in which the main objective of the paper is to determine the metabolism of mutant of *R. oryzae* AS 3.3461. However, the analysis on L-lactic acid production was included in the study. Food waste which was obtained from kitchen refuse was reported by Li et al. [52](S41). They discussed the optimization process of several parameters such as seed culture time, inoculation size and pH of fermentation medium for lactic acid production by *R. oryzae* AS 3.819. Although all these types of substrates have not been studied intensively, they provide more opppurtunities for further investigation and potential since all the reported studies are published in recent years.

3.3.8. Mixed Substrates

Two out of three studies reported the use of mixed glucose and xylose. A study by Zhang et al. [53](S21) used a mixture of glucose and xylose in a synthetic medium to investigate the effects of carbohydrate-degradation products on metabolic activity of *R. oryzae* especially lactate dehydrogenase (LDH) and alcohol dehydrogenase (ADH), cell growth and L-lactic acid and ethanol production. Besides studying the effect of xylose alone, Saito et al. [33] (S51) also used a mixture of glucose and xylose to evaluate the proficiency of *R. oryzae* NBRC 5378 to convert the substrate into lactic acid. The only study that investigates the D-lactic acid production by *Rhizopus sp* WS0128 was done by Shen and Wang [54](S55) where the use of glucose and sucrose at the same concentration was added into the fermentation medium.

3.4. Raw Starchy Materials State of Origin

Sub-RQ 1: Which Country focuses on raw starchy materials as substrate to produce lactic acid by *Rhizopus sp*?

Table 4 shows the list of studies that used raw starchy materials and its state of origin and lactic acid production. Out of 32 studies, only 11 studies used raw starchy materials as a substrate. Most of the studies are from the United states while three of the studies arefrom Asia region and one from the United Kingdom.

Refferring to study no S24 in Table 4, the authors mentioned that they obtained inedible cassava tubers and leaves from a local plantation in Malaysia and from the three bibliographic databases, this is the only study that highlighted the utilization of inedible cassava. One study featured using sweet potato in the fermentation process which is S58 where a species of Taiwan potato was bought from a local supermarket. S88, S153 and S158 investigated rice as carbon source for lactic acid fermentation. Both S88 and S158 grounded the rice into powder to be added into the fermentation medium, while S153 used the rice liquid as the medium. All the stated studies did not specifically mention the source of obtaining the raw materials, thus the state of origin is assumed to be the same country as the location of the university of the author(s).

Table 4: List of studies that used raw starchy materials and its state of origin and lactic acid production

Study no.	Raw materials	State of origin	Max. lactic acid yield (w/w%)
S24	Inedible cassava tubers and leaves	Malaysia	91.2
S58	Sweet potato	Taiwan	73
S88	Rice grain	India	75
S90	Oat	United Kingdom	68
S105	Cull potatoes	United States	53
S121	Sorghum	United States	25
S124	Sorghum	United States	31
S153	Rice	United States	61.5
S158	Barley, corn, oats, rice, cassava	United States	±27.5 (barley and cassava), ±42.5 g/kg (corn and rice) and ±11.0 (oat)
S159	Corn	United States	39.0
S161	Sorghum	United States	16.5

Two studies used oats in their research which are S90 and S158. The oats used by S90 were supplied by an oat company located in the United Kingdom while no location was mentioned in S158. The use of potato and corn as substrates are very common especially potato starch and corn starch. However, only one of the listed studies investigated the utilization of cull potatoes and two studies used corn in lactic acid production. The studies are S105 for cull potatoes and S158 and S159 for corn. The source of the cull potatoes was not introduced in the methodology section, but the authors briefly described a tribute of potatoes crop as a major crop in Pacific Northwest especially Washington, Oregon and Idaho [28]. Procurement of corn in S159 was not stated as well. Three studies which are S21, S123 and S161 describe the use of sorghum, sort of cereal grain in their experiments. All mentioned studies introduced sorghum as the one the major crops planted in the United States and ranks third among the cereal crops [55-57].

Sub-RQ2: What is the production yield of the lactic acid identified from Sub-RQ1?

Table 4 also includes maximum lactic acid yield obtained from each study. Half of the studies produced above 50 w/w% of lactic acid. While sorghum and corn recorded lower yields of lactic acid. The yield of lactic acid produced depends on the fermentation conditions and parameters applied in the studies. In addition, several studies conducted shake-flask fermentation while some of it in the bioreactors. Thus, the range of yield and concentration of lactic acid are greatly varied. Also, every raw starchy material has different chemical constituents such as carbohydrates, proteins, fatty acid, and minerals. These varying chemical compositions of raw starchy materials are the factor that affect lactic acid production especially carbohydrate content. Besides, determining the optimum concentration of the substrates in fermentation medium is also important in lactic acid production despite high carbohydrate contents in raw materials. Other than that, the minerals contained in raw materials sometimes supplied sufficient nutrients in the fermentation medium that will cause the addition of several nutrients has no effect on the production of lactic acid or inhibit the production.

4. CONCLUSIONS

The network visualization and the classification of substrates according to its types were successfully performed through mapping literature study. Three bibliographic databases were employed in this study: PubMed, Scopus and Dimensions. Among the keywords extracted, glucose and lactic acid shows a strong and importance connection between each other indicating the researchers and scientists prefer to use glucose as the substrate. Additionally, several similar keywords were found to intersect with each other. Eleven types of substrates which are glucose, starch, lignocellulosic materials, xylose, glycerol, different carbohydrates, microalgae, seaweeds, gluconic acid, food wastes and mixed substrates, were identified after grouping and classifying the studies accordingly. Glucose was found to be the most used substrate for lactic acid production followed by lignocellulosic materials and starch. The most frequent origin of starch are corn, potato and cassava. Less studies reported on the use of raw starchy materials in the fermentation process. Most of the studies are reported from the United States and only three studies are from Asia. The overview presented in this study could help the researchers to dig into the areas that are yet to develop or mature. Due to the growing demand of lactic acid, efficient and sustainable susbstrate are sought after. This study has mapped the most popular substrate in the research field. The least interested substrates such as glycerol, mixed substrates and food waste have various approaches that can be implemented for future research. For examples, to study lactic acid production by using immobilized cells, the effects of cells morphology or different parameters on lactic acid production is worth to explore. Thus, a new hypothesis can be made from these approaches.

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APPENDIX A

List of included studies. The references listed in Table A.1 correspond to those prefaced with the letter "S" throughout the paper.

Table A.1: List of studies from PubMed, Scopus and Dimensions.

Study no	References
S1	Zain, N. A. M., Aziman, S. N., Suhaimi, M. S., & Idris, A. (2021). Optimization of L (+) Lactic Acid Production from Solid Pineapple Waste (SPW) by Rhizopus oryzae NRRL 395. Journal of Polymers and the Environment, 29(1), 230-249.
S2	Yin, L., Luo, X., Zhang, Y., Zheng, W., Yin, F., & Fu, Y. (2020). Comparative proteomic analysis of Rhizopus oryzae hyphae displaying filamentous and pellet morphology. 3 Biotech, 10(11), 1-10.
S3	Ma, X., Gao, M., Yin, Z., Zhu, W., Liu, S., & Wang, Q. (2020). Lactic acid and animal feeds production from Sophora flavescens residues by Rhizopus oryzae fermentation. Process Biochemistry, 92, 401-408.
S4	Shahri, S. Z., Vahabzadeh, F., & Mogharei, A. (2020). Lactic acid production by loofah-immobilized Rhizopus oryzae through one-step fermentation process using starch substrate. *Bioprocess and biosystems engineering*, *43*(2), 333-345.
S5	Dhandapani, B., Vishnu, D., Murshid, S., & Sekar, S. (2021). Production of lactic acid from industrial wastepaper sludge using Rhizopus oryzae MTCC5384 by simultaneous saccharification and fermentation. *Chemical Engineering Communications*, *208*(6), 822-830.
S6	Maslova, O., Stepanov, N., Senko, O., & Efremenko, E. (2019). Production of various organic acids from different renewable sources by immobilized cells in the regimes of separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SFF). *Bioresource technology*, *272*, 1-9.

S7	Yuwa-Amornpitak, T., & Chookietwatana, K. (2018). Bioconversion of waste cooking oil glycerol from cabbage extract to lactic acid by Rhizopus microsporus. *brazilian journal of microbiology*, *49*, 178-184.
S8	Matsumoto, M., & Furuta, H. (2018). In situ extractive fermentation of lactic acid by Rhizopus oryzae in an air-lift bioreactor. *Chemical and Biochemical Engineering Quarterly*, *32*(2), 275-280.
S9	Chen, X., Wang, X., Xue, Y., Zhang, T. A., Li, Y., Hu, J., ... & Gao, M. T. (2018). Influence of rice straw-derived dissolved organic matter on lactic acid fermentation by Rhizopus oryzae. *Journal of bioscience and bioengineering*, *125*(6), 703-709.
S10	Fu, Y., Sun, X., Zhu, H., Jiang, R., Luo, X., & Yin, L. (2018). An optimized fed-batch culture strategy integrated with a one-step fermentation improves l-lactic acid production by Rhizopus oryzae. *World Journal of Microbiology and Biotechnology*, *34*(6), 1-8.
S11	Cui, Y., Liu, R., Xu, L., Zheng, W., & Sun, W. (2018). Fermentation kinetics of enzymatic hydrolysis bagasse solutions for producing L-lactic acid. *Sugar Tech*, *20*(3), 364-370.
S12	Zhang, J., Guo, Z., Liu, B., Wang, Y., Yu, N., & Huo, Q. (2018). Optimization of L-lactic acid production by fermentation of Huaimi dregs. *Biotechnology & Biotechnological Equipment*, *32*(2), 357-365.
S13	Trakarnpaiboon, S., Srisuk, N., Piyachomkwan, K., Yang, S. T., & Kitpreechavanich, V. (2017). L-Lactic acid production from liquefied cassava starch by thermotolerant Rhizopus microsporus: characterization and optimization. *Process Biochemistry*, *63*, 26-34.
S14	Panesar, P. S., & Kaur, S. (2017). Screening of media components and process parameters for production of L (+) lactic acid from potato waste liquid using amylolytic Rhizopus oryzae. *Acta Alimentaria*, *46*(3), 312-322.
S15	Pimtong, V., Ounaeb, S., Thitiprasert, S., Tolieng, V., Sooksai, S., Boonsombat, R., ... & Thongchul, N. (2017). Enhanced effectiveness of Rhizopus oryzae by immobilization in a static bed fermentor for l-lactic acid production. *Process Biochemistry*, *52*, 44-52.
S16	Takano, M., & Hoshino, K. (2016). Lactic acid production from paper sludge by SSF with thermotolerant Rhizopus sp. *Bioresources and Bioprocessing*, *3*(1), 1-10.
S17	Fu, Y. Q., Yin, L. F., Zhu, H. Y., & Jiang, R. (2016). High-efficiency l-lactic acid production by Rhizopus oryzae using a novel modified one-step fermentation strategy. *Bioresource technology*, *218*, 410-417.
S18	Zheng, Y., Wang, Y., Zhang, J., & Pan, J. (2016). Using tobacco waste extract in pre-culture medium to improve xylose utilization for l-lactic acid production from cellulosic waste by Rhizopus oryzae. Bioresource technology, 218, 344-350.
S19	Maslova, O. V., Sen'ko, O. V., Stepanov, N. A., & Efremenko, E. N. (2016). Lactic acid production using free cells of bacteria and filamentous fungi and cells immobilized in polyvinyl alcohol cryogel: A comparative analysis of the characteristics of biocatalysts and processes. *Catalysis in Industry*, *8*(3), 280-285.
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