Review Article

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Use of glycerol waste in lactic acid bacteria metabolism for the production of lactic acid: State of the art in Poland

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Abstract: Lactic acid is a naturally existing organic acid, which may be used in many different branches of industrial application. It can be made in the sugar fermentation process from renewable raw lactic acid, which is an indispensable raw material, including in the agricultural, food, and pharmaceutical industries. It is an ecological product that has enjoyed great popularity in recent years. In 2010, the US Department of Energy published a report about lactic acid to be a potential building element for future technology, whose demand grows year by year. The lactic acid molecule naturally exists in plants, microorganisms, and animals and can also be produced by carbohydrate fermentation or chemical synthesis from coal, petroleum products, and natural gas. In industry, lactic acid can be produced by chemical synthesis or fermentation. Although racemic lactic acid is always produced chemically from petrochemical sources, the optically pure L(+) – or D(–) – lactic acid forms can be obtained by microbial fermentation of renewable resources when an appropriate microorganism is selected. Depending on the application, one form of optically pure LA is preferred over the other. Additionally, microbial fermentation offers benefits including cheap renewable substrates, low production temperatures, and low energy consumption. Due to these advantages, the most commonly used biotechnological production process with the use of biocatalysts, i.e., lactic acid bacteria. The cost of raw materials is one of the major factors in the economic production of lactic acid. As substrate costs cannot be reduced by scaling up the process, extensive research is currently underway to find new substrates

for the production of LA. These searches include starch raw materials, lignocellulosic biomass, as well as waste from the food and refining industries. Here, the greatest attention is still drawn to molasses and whey as the largest sources of lactose, vitamins, and carbohydrates, as well as glycerol – a by-product of the biodiesel component production process. Focusing on the importance of lactic acid and its subsequent use as a product, but also a valuable raw material for polymerization (exactly to PLA), this review summarizes information about the properties and applications of lactic acid, as well as about its production and purification processes. An industrial installation for the production of lactic acid is only planned to be launched in Poland. As of today, there is no commercial-scale production of this bio-raw material. Thus, there is great potential for the application of the lactic acid production technology and research should be carried out on its development.

Keywords: lactic acid, lactic acid bacteria, waste management, glycerol, circular economy

1 Introduction

Circular economy (CE) is the trend in engineering that improves the development of technology to maximize the exploitation of resources and make them recyclable [1–3]. This tendency is observed also in fuels and energy management, especially in renewable resources of fuels and energy [4]. The term circular economy covers the steps of recycling in which technology should preserve the resources or prevent waste production; design of product making possible to reuse parts of the product, possibly the whole product; and recycle material or finally (and at least) energy recycling of flammable residues [5–8]. The last opportunity could be widened by the other technologic options like gasification of organic residues to a kind of syngas and use it for energy production in a fuel cell [9–11]. Increasing attention to renewable energy resources is given in recent years, among others to the

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production of renewable liquid fuels. One significant example of fermentation usage is the production of alcohols and to further extend ethers as a substitute (or add-on) of gasoline in tropical and mild climate regions, respectively [12–17]. Even etherification of glycerol is regarded as a possibility for automotive renewable fuel production [18,19]. Anaerobic digestion is used for biogas production as a biofuel but possibilities of its usage are significantly higher in biofuel production and by-products utilization [20–24]. Many strategies for a circular economy are met in engineering and management. Thus, recovery of additional commercial goods from the process of organics production (among other fuels) is an important prospect in the point of view of the circular economy [25–28]; in the focus of the authors are bioprocesses used in renewable fuel production. Some of those processes are waste-less or with minimal by-product generation (e.g., the conversion of bioethanol to bioether) but in some other processes by-products generation is significant (e.g., fatty acids conversion to FAME with glycerol formation). Waste biomass is transformed into many commercial products by several physicochemical or biological processes applying the rules of green chemistry [29]. The first step is to recover the secondary raw materials. Next, the most appropriate way for biomass recycling is the refining of secondary biocomponents for further processes and then using other steps of

recycling. Figure 1 illustrates the idea of biorefinery for waste biomass.

Boldface arrows on the chart (Figure 1) represent primary processes of biomass wastes or by-product treatment while the slender ones describe secondary processes. Both bioprocesses and classic physicochemical treatment are used in bio-refining or fuels and energy recovery. In biotechnology, fermentation plays a special role and often is associated with hydrolysis as preprocessing [30,31]. Fermentation leads to the production of bioethanol [13,32,33], biogas [20,21,34,35], hydrogen from dark fermentation [36,37], as well as biomass reforming for fuel cell purpose [38]. In classic physicochemical processes, preliminary processing leads to syngas, and in secondary processes hydrocarbons are produced by catalytic synthesis [39,40]. Moreover, syngas could be used as a substrate for fermentation toward alcohols and organic acids [41,42]. Another classic processing process is pyrolysis, which could yield solid carbonizate – char, pyrolytic gases, and pyrolysis oil containing a mixture of organic compounds. Char has possible application as an adsorbent, pyrolytic gases as fuel, and some chemicals could be separated from pyrolysis oil (e.g., limonene) or hydrocarbons are produced by hydrotreating [43–45].

Finally, the focus of this paper is the processing of biofuels, specially FAME. Glycerol is a by-product of the transesterification of fatty acids [46]. In the classic

Figure 1: The idea of components or energy recovery from bioprocesses waste or by-product. Descriptors: (1) fermentation, (2) hydrolysis, (3) pyrolysis, (4) outgasification, (5) combustion, (6) reforming (hydro-reforming), and (7) catalytic synthesis.

approach, the alkali catalyst is used but recent trends are to use enzymes, e.g., lipase [47]. Such an approach makes the process more renewable and eco-friendly because all substrates are from biological sources. The aim is to make the process even more efficient and eco-friendly at every stage of the processing of main and by-products. One of the possible solutions that are currently being considered is the biorefining of glycerol as a by-product of biocomponent production for diesel fuel [48,49].

Some value-added chemicals could be produced from glycerol, among others: succinic acid [50], hydrogen in the hydrothermal conversion of acrolein [51], and lactic acid [52]. In particular, the role of lactic acid has been growing in recent years in many applications ranging from food processing, through cosmetics, medicine, and ending with typically engineering applications such as the production of lactic acid polymer (PLA) and its subsequent use as a biodegradable additive to other materials, production of filaments for printers, and 3-D and prototyping various goods [6,53,54].

2 Catalytic methods of lactic acid production from glycerol

Scientists or engineers use different strategies for lactic acid production from glycerol applying chemical reactions with different kinds of catalysts or biotechnology methods utilizing microorganisms of a different kind, including GMO ones. Komanoya et al. propose a combined catalyst with Pt nanoparticles and TiO₂ as an effective improvement of reaction with 63% yield [55]. Yin et al. elaborated graphite-based catalyst with NiO nanoparticles, which improve the selectivity of reaction up to 97.6% in an alkaline water solution of glycerol [56]. Another team with the same leader (Yin-Zhang et al.) developed a series of Cu-based catalysts (Cu/hydroxyapatite, Cu/MgO, Cu/ZrO₂) resulting in 91% conversion of glycerol and 90% selectivity toward lactic acid [57]. A similar idea followed the team of Moreira et al.; they produced a series of catalysts using copper and based on Al₂O₃, ZnO, or MgO also in an alkaline glycerol solution [58]. The team of Arcanjo et al. tested an activated carbon catalyst with noble metals deposited on 5% Pt/C and 10% Pd/C giving 99% glycerol conversion with 68 and 74% selectivity, respectively [59]. Noble metals were used as active material on activated carbon by Zhang et al. with mild alkali solution; LiOH, KOH, NaOH, and Ba(OH)₂ were tested and they conclude that many variants of conditions play role in the conversion to lactic acid with selectivity

from 69.3 to 100% [60]. Yang et al. developed zirconia-supported copper oxide catalysts (CuO/ZrO₂) and depending on the composition they even obtained 100% glycerol conversion with 94.6% selectivity to lactic acid [61]. A different approach was presented by Rodrigues et al. also using an alkaline medium for conversion, while NaOH and KOH were used as catalysts and the reaction reached 97% efficiency [62]. Only selected technologies for the catalytic conversion of glycerol to lactic acid are presented above. The latest research introduces further modifications to the catalysts: Cu on CaO/MgO [63], zirconium–cerium oxides/SBA-15 [64], Au/bentonite [65], Ni–NiO_x [66], Au/hydroxyapatite/BN [67], and research is still in progress.

All chemical methods of converting glycerol have a common disadvantage of the necessary energy input since most of the reactions were carried out at elevated temperatures. Biotechnological methods do not require additional energy for the reaction and are carried out under moderate temperature conditions. Unfortunately, the degrees of glycerol conversion in biochemical methods are usually much lower, and at the same time require glycerol dilutions. However, in the point of view of green chemistry, biotechnological processes of glycerol conversion are more environmentally friendly [29].

However, a review of biotechnology methods for glycerol conversion to lactic acid is the purpose of this publication and is presented in the following section.

3 Lactic acid production by biotechnological methods

The chemical synthesis of the commercial process is based on lactonitrile and this process occurs in the liquid phase at high atmospheric pressure [68–70,73]. This process has many steps to produce lactic acid and is represented by the following reactions:

A chemical reaction to lactic acid production.

This chemical synthesis yields a racemic mixture of lactic acid. Musashino, Japan, and Sterling Chemicals

Inc., USA, are using this technology on the fabric procedure. The next options are base-catalyzed degradation of sugars, the reaction of acetaldehyde, oxidation of propylene glycol, carbohydrate fermentation, and many others [84,85,90].

Fermentation is the best option to provide an ecological and economical biotechnology process. A batch reactor, a half-batch reactor, and a reactor with repeated dosing of feedstock, and a continuous reactor are the most frequently used reactors in the production of lactic acid. The higher concentration of lactic acid has been tested and is obtained in cultures using a batch reactor and semi-batch reactor, while higher productivity is obtained by a continuous reactor. Reports in the literature state that the latest biotechnological research on lactic acid differs in fermentation and process methods and process parameters [71,72,82,83].

The parameters responsible for the fermentation are temperature, flow rate, pressure, mixing, pH, and oxygenation [78–81]. These factors are the most important and of great influence to process because they indicate productivity, selectivity, and yield of a reaction [77,89].

The most important, however, is the selection of microorganisms for the fermentation process, which may affect the technological regime and the profitability of the process. Currently, many microorganisms useful for the production of lactic acid have already been identified, and further applications and optimization of current processes are also being worked out [86–88]. Exemplary cultures of microorganisms used in the production of lactic acid are presented in Table 1.

Various substrates are taken into account for the production of lactic acid. Screening shows that for a given substrate, a specific microorganism or a set of microorganisms

can be selected for optimal processing of the raw material into lactic acid. The choice of substrates with selected microorganisms is presented in Table 2.

The use of alternative substrates in fermentation processes, aiming at the utilization of agricultural low-cost raw materials or by-products from various industries (molasses, bran, corn syrup, whey, etc.) lowers the cost of the culture medium used and hence the final product [74,75,91]. However, these substrates have complex compositions whose exact total is often unknown. In addition to the carbon source and other nutrients, some compounds that may be present or even formed during the process steps, as pre-treatment, may be factors capable of inhibiting the growth of microorganisms or prevent the synthesis of the metabolite of interest [70,92,97].

The most commonly used substrate for *Lactobacillus* (L.) *rhamnosus* ATCC 10863 fermentation for lactic acid production is glucose, but cellulose, lignocellulose, and sucrose are also used. Molasses hydrolyzed can also be used and, in this work, the lactic acid production with this cheap and green substrate without a pre-treatment will be explored [76,77,95,96,98].

Along with the growing interest in biocomponents for diesel fuels, a large amount of glycerol appears in the market. It is used in many industries, as an additive to cosmetics or as fuel. An interesting direction in the processing of this by-product would be its transformation into lactic acid as a chemical raw material. Currently, there is little information in the literature on the biotechnological conversion of glycerol to lactic acid, and hence, the authors' interest in the development and optimization of this method. In particular, in Poland, there will also be an increase in the production of biocomponents, and thus glycerol, as a result of the adjustment to the EU policy.

Table 1: Example of yield, productivity, and quantity of lactic acid microbial production [83]

Microorganism	Concentration (g/L)	Yield (g/g)	Productivity (g/L h)
<i>Rhizopus oryzae</i> ATCC 52311	83.0	0.88	2.6
<i>Rhizopus oryzae</i> NRRL 395	104.6	0.87	1.8
<i>Enterococcus faecalis</i> RKY1	144.0	0.96	5.1
<i>Lactobacillus rhamnosus</i> ATCC 10863	67.0	0.84	2.5
<i>Lactobacillus helveticus</i> ATCC 15009	65.5	0.66	2.7
<i>Lactobacillus bulgaricus</i> NRRL B-548	38.7	0.90	3.5
<i>Lactobacillus casei</i> NRRL B-441	82.0	0.91	5.6
<i>Lactobacillus plantarum</i> ATCC 21028	41.0	0.97	1.0
<i>Lactobacillus pentosus</i> ATCC 9041	21.8	0.77	0.8
<i>Lactobacillus amylopilus</i> GV6	76.2	0.70	0.8
<i>Lactobacillus delbrueckii</i> NCIMB 8130	90.0	0.97	3.8
<i>Lactococcus lactis</i> ssp. <i>Lactis</i> IFO 12007	90.0	0.76	1.6

Table 2: Examples of cheap and popular waste material for feedstock application in producing lactic acid biotechnologically [83]

Substrate	Organism	Concentration (g/L)	Productivity (g/L h)
Molasses	<i>Lactobacillus delbrueckii</i> NCIMB 8130	90.0	3.8
	<i>Enterococcus faecalis</i> RKY1	95.7	4.0
Rye	<i>Lactobacillus paracasei</i> No. 8	84.5	2.4
Sweet sorghum	<i>Lactobacillus paracasei</i> No. 8	81.5	2.7
	<i>Lactobacillus paracasei</i> No. 8	106.0	3.5
Wheat	<i>Lactococcus lactis</i> ssp. <i>lactis</i> ATCC 19435	106.0	1.0
	<i>Enterococcus faecalis</i> RKY1	102.0	4.8
Corn	<i>Enterococcus faecalis</i> RKY1	63.5	0.5
	<i>Lactobacillus amylovorus</i> ATCC 33620	10.1	0.8
Cassava	<i>Lactobacillus amylovorus</i> ATCC 33620	4.8	0.2
Potato	<i>Lactobacillus amylovorus</i> ATCC 33620	4.2	0.1
	<i>Lactobacillus</i> sp. RKY2	129.0	2.9
Rice	<i>Lactobacillus casei</i> NRRL B-441	162.0	3.4
Barley	<i>Lactobacillus amylophilus</i> GV6	27.3	0.3
Cellulose	<i>Lactobacillus coryniformis</i> ssp. <i>torquens</i> ATCC 2560	24.0	0.5
	<i>Rhizopus</i> sp. MK-96-1196	24.0	0.3
Corncob waste paper	<i>Lactobacillus coryniformis</i> ssp. <i>torquens</i> ATCC 2560	23.1	0.5
	<i>Rhizopus orizae</i> NRRL 395	49.1	0.7
	<i>Lactobacillus delbrueckii</i> NRRL B-445	108.0	0.9
Wood	<i>Enterococcus faecalis</i> RKY1	93.0	1.7
	<i>Lactobacillus helveticus</i> R211	66.0	1.4
Whey	<i>Lactobacillus casei</i> NRRL B-441	46.0	4.0

4 The potential of lactic acid production from biofuel by-product in Poland

Poland produces about one million tonnes of biodiesel annually in 2020 and will increase significantly until 2030. In addition, Poland is expected to increase from 10% under the biodiesel obligation to 20% by 2030. An increase in biodiesel production is therefore related to inevitable abundance of glycerol as by-product. That increased amount of crude glycerol must be used for increasing efficiency of biodiesel industry by introducing new pathways for circular economy, e.g., lactic acid production. Consequently, almost the entire industry uses only refined glycerol as raw material, as unrefined glycerol has become a potential environmental pollutant. Purification of glycerol is a much more costly process, and the low process level obtained recently made it economical. For the sustainability of the biodiesel industry, it is important to find a viable and efficient solution to converting waste glycerol into valuable products. Alternative oil handling solution of waste glycerol is transforming it into a more valuable product, for example, lactic acid [72,99,100,105].

In Poland, over a million tonnes of biofuels are already used. They help to reduce emissions in transport, and is expected to grow in the coming years. The share of

renewable energy in Polish transport is not optimistic – it is far from the requirements of 2020 and lower than the EU average, mainly due to the fact that Poland probably will not meet the EU renewable energy target for later years. But, the result only improves with biofuels. Over one million tons of biofuels were used with almost 25 million tons of total official consumption of liquid fuels. Importantly, the use of “advanced” biofuels, for example, from waste, which counts twice in EU statistics, has finally increased [109,110].

According to the data from Trend Economy about open access to data on the import and export of molasses and glycerol as raw materials in the production of lactic acid, the information is presented in Table 3.

The value of glycerine exports from Poland shows the large possibilities of processing this by-product. The balance of exports against imports shows the overproduction of glycerol, which could be transformed into other valuable products, e.g., lactic acid.

Two years back, it turned out that the share of renewable energy sources in transport, instead of growing was decreasing. This is due to the fact that after the elimination of the gray economy, fuel consumption increased rapidly, which did not translate into biofuels. In 2018, however, something changed. The share of biofuels in transport increased by more than a half, and the share of renewable energy sources was 5.63% – the Central Statistical Office reported in 2018 [93,101,103].

Table 3: Import and export of glycerol in the world (chosen country) in 2019 [115]

Country	Glycerol, glycerol waters, glycerol lyes			
	Exports		Imports	
	Value (US\$)	World share (US\$)	Value (US\$)	World share (US\$)
Indonesia	123,269,715	26.97	881,140	0.15
Brasil	48,776,123	10.67	79,709	0.01
Spain	35,369,146	7.73	2,606,929	0.44
Germany	33,369,146	7.32	44,083,259	7.57
France	30,175,005	6.60	2,099,318	0.36
Malaysia	22,992,707	5.03	22,766,139	3.91
Netherlands	20,894,871	4.57	34,310,242	5.89
USA	18,754,794	4.10	9,296,641	1.59
Argentina	12,691,168	2.77	1,587	0.00
Poland	12,649,298	2.76	343,016	0.05
Belgium	12,524,594	2.74	13,917,475	2.39
Thailand	10,066,763	2.20	1,334,636	0.22
Portugal	7,064,742	1.54	127,995	0.02
Canada	6,086,080	1.33	3,328,951	0.57
United Kingdom	5,375,405	1.17	17,869,989	3.07
Colombia	5,330,001	1.16	8,091	0.00
Korea	5,233,883	1.14	1,477,898	0.25
Czech Republic	4,637,885	1.01	5,264,982	0.90
India	4,057,010	0.88	23,110,723	3.97
Romania	4,013,475	0.87	109,713	0.01
Lithuania	3,612,292	0.79	75,553	0.01
Greece	3,498,359	0.76	28,357	0.00

Note: Bold row in Table signs the situation in Poland as a country regarded in the title of paper.

Nowadays, the market of the biofuels industry and waste management is huge. The policy of the fuels market is complicated but the market forecasts show quite good statistics of the growing demand for biofuels, and hence, the growing waste market.

Optimized, effective waste management is integral to petroleum refinery operations. It helps minimize risk to both people and the environment, enhances resource utilization, and can also reduce costs. Many countries have detailed legislative requirements and control systems that apply to all aspects of waste management, while others have less regulatory oversight and guidance [69,94,103,104].

Petroleum refineries generate one of the most important categories of waste – process waste. Refineries produce industrial process wastes that are inherent to the activities they carry out in the handling and processing of crude petroleum and petroleum products [102,106,107].

5 Investigated method for use of LAB

A few studies by Polish scientists so far have not shown the daily calculations and the transformation into large-scale technology. Research groups are investigating this matter; however, it is a fresh topic in Poland. Additionally, numerous worldwide studies show how important is the subject of ecological changes.

The world production of lactic acid is estimated at around 520 thousand tons per year. So far, lactic acid has not been produced in Poland, and its shortage in the entire region of Central and Eastern Europe forced the recipients of this raw material to import from Asia and Western Europe [93,108].

A project led by Polish researchers implement the first installation in Poland operating on the basis of an innovative technology for the production of lactic acid using waste from agri-food and biorefinery industry. Lactic acid has a key role in the production of the most popular, fully biodegradable polymer, polylactide (PLA), used among others for the production of biodegradable packaging. PLA has a number of applications in the construction industry, technology, optics, and the automotive industry, and due to its properties, including transparency, it is also used in the production of photovoltaic cells, as well as in medicine and pharmacy, where it is used for the production of implants, screws, and surgical threads [111–114].

First of all, lactic acid bacteria metabolism has to be studied. The research plans include screening the microorganisms for the best, most economical bacteria as a biocatalyst. In addition, the catalyst used in the biotechnological process should have a low-cost financial outlay, i.e., process parameters. To judge this, a series of studies must be conducted showing possible pathways to the process. Subsequently, the fermentation process itself, first on a laboratory scale, and then in large-scale production with the proposed purification process will be conducted. According to world studies, the purification stage is the most expensive. However, to sum up, and taking into account the advantages and disadvantages – knowing that waste can be used, the treatment process can be covered by the lower energy consumption of the process than the one carried out with the chemical method.

We are currently testing three LAB species in our laboratory: *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Lactobacillus leichmanii*. Preliminary studies show great difficulty in cultivating any LAB species on

Figure 2: Import and export Polish molasses in 2008, 2015, and 2019 (from left) [116].

pure glycerin. Therefore, we decided to mix raw glycerin in a water solution with other waste materials like molasses. The results currently obtained do not yet allow the process to scale, but are promising. The concentration of glycerin in the aqueous solution must not exceed 5%; otherwise, there is a strong toxic effect on LAB. We tested various concentrations of glycerol up to 5% in solutions, as well as other natural waste substances, e.g., molasses or fruit peels. There is an increase in the concentration of lactic acid and a decrease in the concentration of glycerol. Lactic acid concentration is measured by using an iron chemical complex and UV-Vis spectrophotometer.

The use of molasses as a co-reactant is justified as it is also a waste material with high availability, especially it is also a precursor for the production of glycerin by chemical means. The presence of sugar-rich molasses in a mixture with glycerin should increase the efficiency of the lactic acid fermentation process, as suggested by preliminary experiments by using food waste or with the addition of molasses. Molasses is a by-product of sugar production. It was chosen by the authors as the second substance for processing into lactic acid for the comparison of processes. Figure 2 presents the change in the molasses trade balance in Poland.

These data show a huge problem with waste management but also an advantage to scientists who care about human resources and petrochemical processes and develop the biotechnology war for molasses transformation into lactic acid.

The glycerin fermentation procedure with the addition of molasses or food waste requires further development. The team plans to publish the results of ongoing experiments soon.

6 Summary

The uses for glycerol waste continue to expand. The above examples show the valuable products obtainable from this process. It is still a research topic that remains largely untapped. An additional aspect is the development of more and more common white biotechnology. In this field of science, microorganisms are used that are involved in the creation of many valuable chemical products. Biotechnological methods largely prevail over chemical methods. The basis is the possibility of avoiding the disadvantages resulting from the use of catalysts or the purification and preparation of the raw material. This significantly reduces the cost of technology and contributes to conscious waste management. Moreover, this pathway stays in accordance with green chemistry or circular economy, where every product is used in an optimized way and sustainable to the environment.

The preliminary results of the team's laboratory test indicate the possibility of using LAB to convert glycerol or molasses to lactic acid, but the procedure still needs to be developed and optimized, first on a laboratory scale, and then also on an industrial scale.

An industrial installation for the production of lactic acid is only planned to be launched in Poland [117]. As of today, there is no commercial-scale production of this bio-raw material. Thus, there is great potential for the application of the lactic acid production technology and research should be carried out for its development.

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Data availability statement: The datasets generated during and/or analyzed during the current study are available in the repository: https://trendeconomy.com/data/commodity_h2/1520, <https://trendeconomy.com/data/h2/Poland/1703>

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