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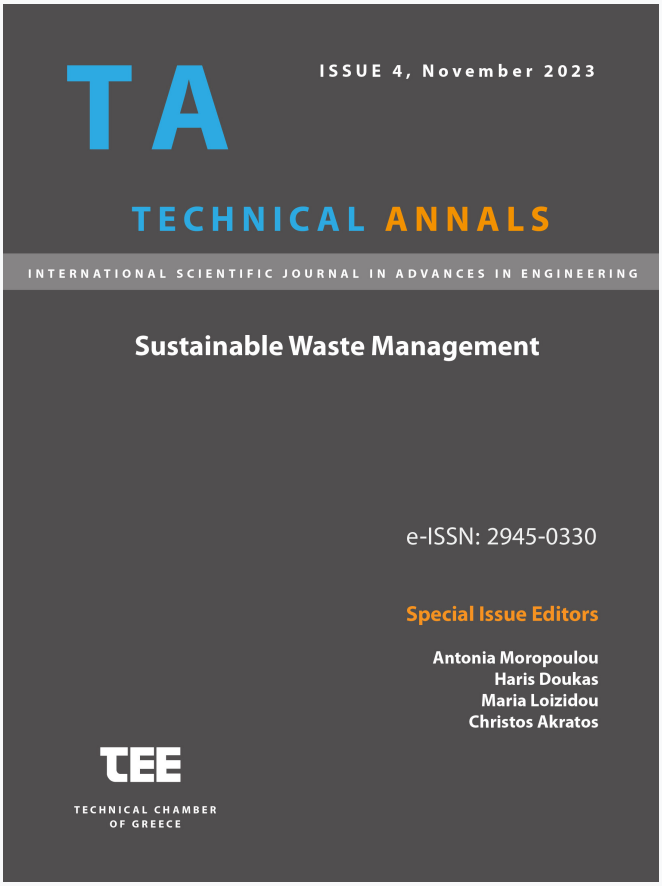

Food industry biowaste as substrate for biosolvents production focusing on lactic acid and ethyl lactate

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Food industry biowaste as substrate for biosolvents production focusing on lactic acid and ethyl lactate

Varvara Karamplia[1], Chrysanthi Stavraki[1], Sofia Mai[0000-0001-9919-8202], Elli Maria Barampouti[0000-0003-4871-8786] and Maria Loizidou[1]

[1]Unit of Environmental Science and Technology, School of Chemical Engineering, National Technical University of Athens 9, Iroon Polytechniou st., 15780 Athens, Greece
mloiz@chemeng.ntua.gr

Abstract. Solvents represent a major category of chemicals due to their necessity in a variety of industries. The majority of solvents derived from fossil carbon are hazardous to both human health and the environment. In this context, it seems imperative to convert biomass into new alternative greener solvents. The present study concentrates on the production of two biosolvents; namely lactic acid and ethyl lactate, from biowaste of baby food industry. For the production of lactic acid parameters as the dosages of enzymes (5 $mg/g_{Lactose}$ and 20 $mg/g_{Lactose}$) and inoculum size (5% v/v and 10% v/v) were examined. The lactic acid concentration reached almost 17.3g/L after 144 hours with $5mg_{LactozymePure}/g_{lactose}$ and 10%v/v inoculum size. Repeated fermentation was also applied yielding a lactic acid productivity of 0.97 g/L h, which is 8 times higher than the conventional batch fermentation. Moreover, the production of ethyl lactate from bioethanol and lactic acid from the fermentation broth was examined. Lactic acid conversion yields as high as 59.9% at 255min were observed. Overall, the results of this work are very promising but further research is needed to optimize conditions and evaluate scalability of the production of these biosolvents from biowaste, since the production of lactic acid and ethyl-lactate from biowaste provides a sustainable approach to the circular economy, limiting waste and minimizing the environmental effect of industrial processes.

Keywords: Biosolvents, Biowaste, Ethyl Lactate, Fermentation, Lactic Acid.

1 Introduction

Solvents are mainly applied in the chemical, food, pharmaceutical, wood and furniture, textile, oil and gas industries, including cleaning processes and chemical formulations. The environmental movement that took place in the 1970s at the United Nations Conference on the Human Environment in Stockholm in 1972 was the landmark of the development of new technologies in the production of solvents. Ever since, environmental, health and safety issues have raised serious concerns about the continuous processing of mineral-based raw materials for the production of chemicals such as fuels and solvents since most solvents are flammable and toxic. Therefore, much effort has been put on reducing the use of hazardous substances (particularly volatile organics)

and on eliminating or minimizing the production of by-products in chemical processes [1]. Green solvents are an emerging renewable alternative to fossil chemicals and have attracted recent research interest [2].

Regarding waste, in the European Union every year, around 7.3 billion tonnes of natural resources are consumed and around 2.7 billion tonnes of waste are produced, of which only 40% is reused or recycled, with the rest going to landfill or incineration. For this reason, in recent years there has been a clear effort to valorize waste, which has increased in quantity, in order to reuse it for energy recovery or for the production of new green products. This effort is very complex and involves several stages. The 2008 revised Waste Directive has established a 5-step hierarchy pyramid, in which prevention is the best option, followed by reuse, recycling, other forms of recovery and finally landfill. According to the new European Circular Economy Strategy, all EU member countries must move to a resource-saving and waste-reusing economy.

The two most common ways of treating organic waste are composting and anaerobic digestion. Composting is the simplest and cheapest process, while anaerobic digestion is best suited to large-scale facilities because of the higher initial cost, but both can be used at any scale [3].

In this paper, the production of lactic acid ($C_3H_6O_3$), which is an organic chemical compound was studied. It finds many applications mainly in the food industry (about 70%) while it is also used in the fields of medicine, cosmetics, animal husbandry, textiles, pharmaceuticals, etc. Lactic acid, classified as a harmless substance by the United States Food and Drug Administration, has a relatively high added value. In addition, many inexpensive materials such as molasses and other residues from agriculture and industry in general have been used as substrates for the fermentation of lactic acid and studies have been conducted to improve the efficiency of microorganisms for the production of lactic acid through gene modification. All of the above makes this solvent ideal for use in industry and particularly capable of becoming one of the key green solvents in the future [4].

The production of lactic acid can be carried out by either chemical or biological processes. The chemical routes are not economically or environmentally viable processes. On the other hand, biotechnological processes (fermentation) have an advantage as they use alternative raw materials as substrates which can be starchy, cellulosic sources or simply organic municipal or industrial waste [5]. Some of the sugars that have been used in the fermentation of lactic acid are glucose, xylose, sucrose, lactose and cellobiose. However, glucose is the most favourable sugar for assimilation by microbial strains for the production of lactic acid. Lactic acid bacteria follow a hierarchical pattern of sugar utilisation, starting with glucose, leading to the suppression of carbon catabolite, which occurs when sugars are not fully utilised, resulting in reduced fermentation efficiency and increased production costs. In order for the fermentation process to take place and produce lactic acid, the presence of microbial strains called lactobacilli (lactic acid bacteria) is required. Lactic acid bacteria (LAB) are a broad, heterogeneous group of common food-grade microorganisms [6]. The nutrient medium is a general-purpose medium used to culture a wide variety of easy-to-use and non-microorganisms, with one of the most effective media being Man, Rogosa, and Sharpe medium (MRS) [7].

In addition to lactic acid, the production of ethyl lactate (EL) was also examined, which is an environmentally friendly and safe chemical substance, mainly produced from biological resources. EL is produced from the esterification reaction of lactic acid and ethanol, particularly from the fermentation of agro-food based materials.

Lactic acid + Ethanol ⇆ Ethyl lactate + Water

It is capable of replacing conventional gasoline-based solvents in many applications, such as paints and cleaners, improving air quality by reducing the amount of carbon dioxide in the atmosphere. Organic esters in general are a very important class of chemicals that have applications in various areas of the chemical industry, such as in perfumes, pharmaceuticals, as plasticisers, as solvents, etc. Almost all manufacturing and processing industries depend heavily on the use of solvents. Some industry experts claim that ethyl lactate could replace traditional solvents in more than 80% of their applications [8]. However, the cost of producing the ester is quite high and as a result it is not yet widely applicable [9].

2 Materials & Methods

2.1 Materials

The substrate used in this study was biowaste generated from the food industry, and more specifically from a single production line, of the baby food sector. This substrate exhibits a substantial content of starch and sugars. It also presents a homogeneous nature and stable composition as a waste material. Analytical methods were employed to scrutinize its initial composition. Moreover, for the production of lactic acid, the amylolytic enzyme Spirizyme EXCEL (40μL/g starch) and the lactase Lactozyme Pure (5 and 20mg/g lactose) were used.

2.2 Analytical Methods

For the estimation of total and water-soluble solids, hemicellulose, cellulose, and lignin in food waste (raw and pretreated), the National Renewable Energy Laboratory (NREL) procedure was applied [10, 11]. For total starch determination, the Total Starch (AA/AMG) test kit (e.g., Megazyme, Wicklow, Ireland) was used (AACC Method 76-13.01). The Soxhlet standard method (5520E) was utilized for the quantification of fats and lipids [[12, 13]. Marketable kits (Glucose oxidase-peroxidase method (GOD/PAP), Biosis SA, Athens, Greece; Spectro-quant Volatile Organic Acids Test 1018909 by Merck KGaA Mellipore, Darmstadt, Germany; Ethanol Assay Kit, K-EtOHLQR, Megazymes) were used for the photometric determination of glucose, volatile fatty acids, and ethanol in the liquid fraction, respectively. Analysis of ethanol, glucose and volatile fatty acids was performed by a high-performance liquid chromatography (HPLC) system equipped with a HyperREZ XP Carbohydrate H+ Counter-ion (8 μm) column. The analysis was carried out at a flow rate of 0.6 mL/min and at 70°C in acidified (0.005M sulphuric acid) ultrapure water as the mobile phase. All analyses took place in triplicate.

2.3 Experimental Methods

Preparation of the fermentation broth for Lactic Acid (nutrient medium MRS and inoculum). The MRS, used as a nutrient medium, after appropriate pretreatment, stood as the substrate for the culture of microorganisms [9]. During its pretreatment, the MRS medium underwent autoclaving at 100°C for 15 minutes. Subsequently, the medium was allowed to cool to a temperature below 40°C to mitigate the risk of microbial degradation. A measured quantity of microorganisms, derived from a pharmaceutical probiotic preparation in powdered form (concentration: 10 mg/mL MRS), was introduced into the culture medium. The resulting solution was then subjected to agitation within a shaking air bath at 37°C and 150 rpm for the required duration stipulated by each distinct experimental procedure.

Production of Lactic Acid. For the purpose of lactic acid production, systematic experimentation was conducted to optimize the process with a focus on enhancing both the concentration of lactic acid generated and the duration of its production (Tables 1-3). Varied parameters were methodically assessed in these experiments to determine the optimal process conditions, while the parameters of load, temperature, volume, and stirring rate remained consistently controlled. All experiments were performed thrice and the results reported are the mean values.

The controlled conditions included a 10% w/w dry solids loading at 37°C, with a reaction volume of 200 mL, and a stirring rate set at 140 rpm. Furthermore, inoculum was introduced in an amount proportional to the load required for each experiment, typically at 5% v/v or 10% v/v, based on the experimental design. At the end of the experiment, a 7.5 mL aliquot was extracted and subjected to a sequential process involving centrifugation, filtration, dilution (D=10), and quantitative analysis.

Table 1. Experimental conditions for testing the effect of lactase.

Experiment	Solid Load (% w/w)	Amylase (μL/g starch)	Lactase (mg/g lactose)	Lactic Acid Bacteria & MRS 24h (% v/v)	T (°C)
1	10	40	5	5	37
2	10	40	20	5	37

Table 2. Experimental conditions for testing the effect of the time of microorganism cultivation.

Experiment	Solid Load (% w/w)	Amylase (μL/g starch)	Lactase (mg/g lactose)	Lactic Acid Bacteria & MRS 24h (% v/v)	Time of microorganism cultivation	T (°C)
3	10	40	5	5	24	37
4	10	40	5	5	48	37

Table 3. Experimental conditions for testing the effect of inoculum size.

Experiment	Solid Load (% w/w)	Amylase (μL/g starch)	Lactase (mg/g lactose)	Lactic Acid Bacteria & MRS 24h (% v/v)	T (°C)
5	10	40	5	5	37
6	10	40	5	10	37

In an effort to increase the productivity of lactic acid, repeated fermentation was also applied examining the addition of enzymes (see Fig. 1). Repeated batch culture usually offers several advantages, including good depletion of medium in the reactor at the end of cultivation, the reuse of microbial cells for subsequent fermentation runs, high cell concentration in the culture, low time required for process operation, and process productivity. Moreover, repeated fermentation achieves greater degradation yields of waste in less time.

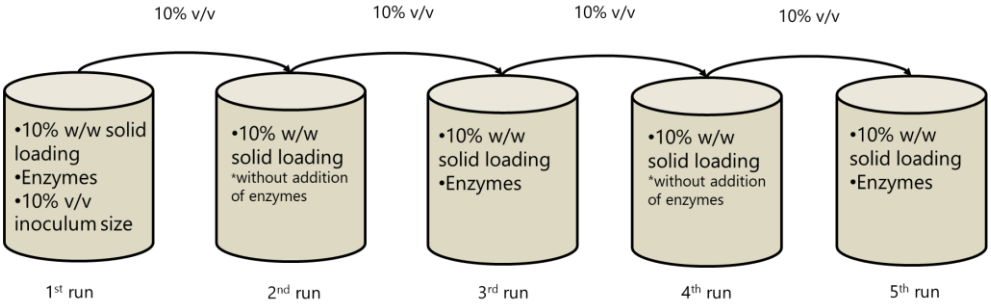

Fig. 1. Experimental protocol for one of the tests conducted during repeated fermentation.

Production of Ethyl Lactate. The synthesis of ethyl lactate, as previously outlined, entails the utilization of lactic acid and ethanol as primary substrates, catalyzing a biphasic esterification reaction. This process generates water as a byproduct. The optimal ethanol-to-lactic acid molar ratio, according to Stipsitz et al. [14], is nEtOH/nLA = 3/1. This ratio was selected due to its ability to curtail the formation of lactic acid oligomers, specifically intramolecular autoesterification, to a minimal extent of approximately 0.4%, contrasting sharply with the 2.4% observed at a 1/1 ratio [14].

An effort to produce ethyl lactate employing bioethanol (98 v/v %) and lactic acid from the fermentation broth as raw materials was also made. The fermentation broth had the necessary concentration of lactic acid, derived from the aforementioned process (0). However, it also included a considerable volume of water, ethanol, and other constituents, thereby exerting notable interference on the esterification reaction. Consequently, pretreatment of the fermentation broth was imperative to mitigate these inhibitory effects.

Upon determination of the lactic acid concentration, the required amount of lactic acid was mixed with an appropriate volume of bioethanol in a flask. The reaction was initiated as soon as Amberlyst 15 catalyst was introduced.

Periodically, samples were extracted from the reaction mixture to monitor the reaction progress. Ethanol was supplemented to the reaction vessel at predefined intervals to counteract ethanol depletion and uphold the initial ethanol-to-lactic acid molar ratio of 3:1.

The trials of ethyl lactate synthesis were performed in a conventional rotary evaporator.

3 Results and Discussion

3.1 Substrate Characterization

By applying analytical methods, the results of the following table (Table 4) for the composition of the waste were obtained.

Table 4. Substrate characterization.

Components	Percentage (%)
Total Solids	99.87±0.16
Moisture	0.13±0.01
Volatile Solids	96.5±0.12
Ash	3.5±0.12
Water Soluble Solids	41.81±0.35
Free Sugars	1.66±0.1
Starch	37±2.26
Oils	2.5±0.43
Total Nitrogen	2.83±0.1
Acid-insoluble residue	5.68±0.45

3.2 Lactic Acid Production

Effect of lactase. Table 5 presents the maximum yields obtained for each experimental condition and the corresponding time intervals. As can be observed from Fig. 2, variations in the lactase enzyme load exhibited negligible influence on lactic acid production over time. However, an observable deceleration in production rate accompanied elevated enzyme loading. Notably, the utilization of 5 mg/g lactose yielded slightly superior production efficiency within a reduced timeframe. Consequently, the use of a lower lactase enzyme load is deemed optimal for lactic acid production, yielding the highest productivity (15± 0.5 g/L within a 168-hour duration).

Table 5. Maximum yields obtained while testing the effect of lactase.

Experiment	Examined parameter	Duration (h)	Yield (%)
1	5mg lactase/g lactose	168	48±1
2	20mg lactase /g lactose	288	51±3

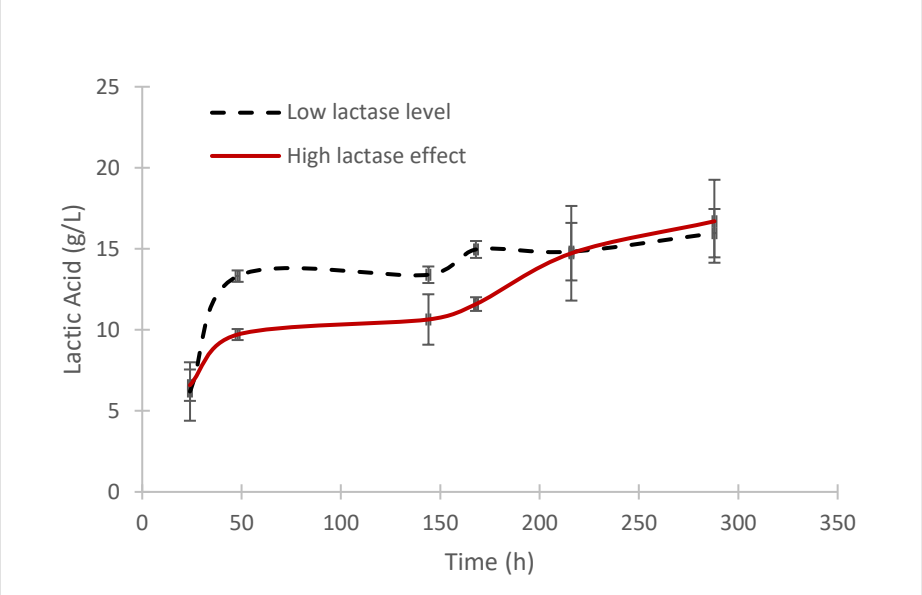

Fig. 2. Effect of lactase dosage on lactic acid production.

Effect of microorganism time cultivation. Regarding the effect of microorganism time cultivation, the maximum yields of each experiment and the times at which they are achieved are shown in Table 6. The different cultivation time of the microorganisms, over time, brought about the same result in the production of lactic acid, i.e. the same concentration was ultimately observed (see Fig. 3). However, a lower production rate was observed in the 48-hour culture, as it took longer to reach maximum concentration 23.85±0.17 g/L than in the 24-hour culture. Therefore, the 24-hour culture of micro-organisms is considered more suitable for optimal production of lactic acid, which also benefits the whole process as it is carried out in a shorter overall time.

Table 6. Maximum yields attained while testing effect of microorganisms time cultivation.

Experiment	Examined parameter	Duration (h)	Yield (%)
3	24h of microorganism cultivation	122	88±1
4	48h of microorganism cultivation	263	74±4

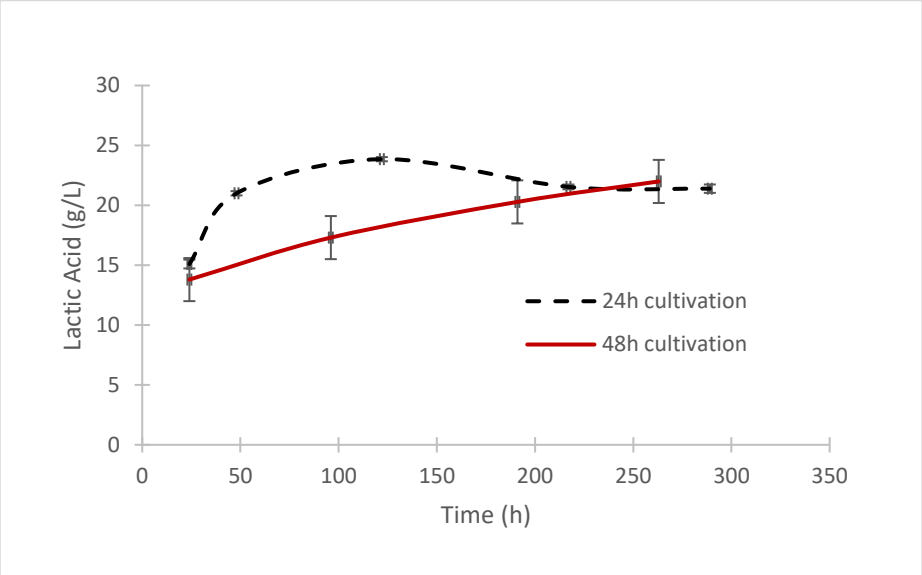

Fig. 3. Effect of microorganism time cultivation.

Effect of inoculum size. As for the effect of inoculum size, the maximum yields of each experiment and the time, in which they were achieved are presented in Table 7. The load of microorganisms significantly affected the production of lactic acid. With the increase of the load, as can be seen in Fig. 4, the concentration of lactic acid increased, with a maximum value of 17.8 ± 0.9 g/L over 288h.

Table 7. Maximum yields attained while testing effect of inoculum size.

Experiment	Examined parameter	Duration (h)	Yield (%)
5	5% v/v Lactic Acid Bacteria & MRS 24h	168	47 ± 4
6	10% v/v Lactic Acid Bacteria & MRS 24h	144	56 ± 3

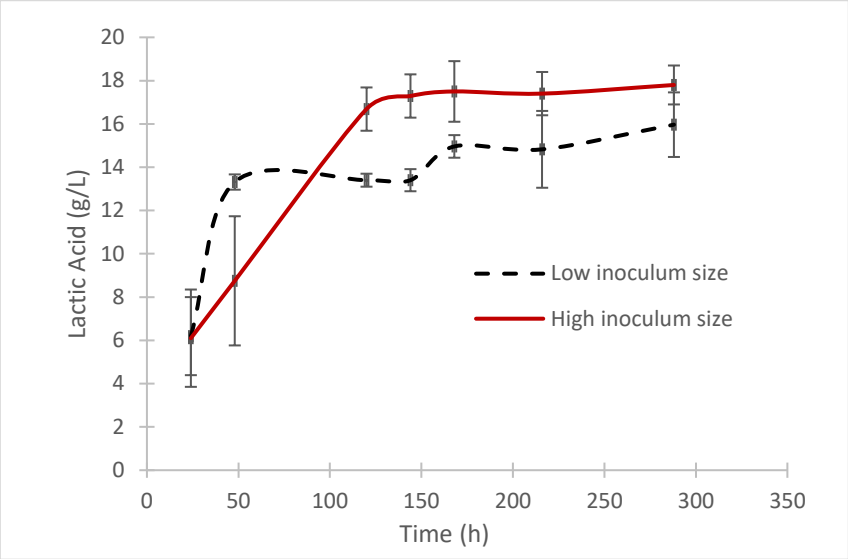

Fig. 4. Effect of inoculum size on lactic acid production.

Repeated Fermentation. Two experimental runs were performed with a microorganism load, 10% v/v but different frequencies of enzyme addition.

In the experiment with alternate enzyme addition, a maximum lactic acid concentration of 24.82 g/L and yield of 87% was obtained in the 4th run as showed in

Table **8**, compared to the maximum lactic acid concentration in the experiment with continuous enzyme addition, which reaches 20.64 g/L and yield 73% in the 3rd run at 48h as showed in Table 9. Thus, adding enzymes alternately in each cycle contributed greatly to the production of lactic acid, increasing the yield. In contrast, the continuous addition of enzymes seems to hamper the process, reducing productivity accordingly.

Table 8. Yields of repeated fermentation adding enzymes alternately.

Run	Duration (h)	Yield (%)
1	72	60
2	72	74
3	48	70
4	24	65
	48	87
5	24	75

Table 9. Yields of repeated fermentation adding enzymes in every run.

Run	Duration (h)	Yield (%)
1	72	72
2	72	70
3	48	73
4	24	43
5	24	56

3.3 Ethyl Lactate Production

Ethyl lactate production by bioethanol and lactic acid from the fermentation broth was achieved, with the maximum lactic acid conversion being 59.9% at 255 min (see Fig. 5**Σφάλμα! Το αρχείο προέλευσης της αναφοράς δεν βρέθηκε.**). Comparable values of 57.5% were achieved at 180 min. The mean lactic acid conversion was 56.2 ±3.2% at 225 ±35min. The water content in the reaction mixture strongly influenced the esterification reaction. Thus, the higher the grade of reactants, the higher the lactic acid conversion.

In general, the production of ethyl lactate using biodegradable solvents poses several challenges primarily due to the nature of lactic acid. The substrate of the present study constitutes a raw material rich in polysaccharides, which, upon water and ethanol removal in the rotary evaporator (lactic acid pretreatment), forms a viscous solution. This solution, detrimental for the reaction with ethanol in the presence of catalyst for ethyl lactate production, becomes even more viscous and dysfunctional over time, rendering it unsuitable for further processing. However, the results are promising although further research is necessary in order to achieve greater conversion of lactic acid and hence production of ethyl lactate.

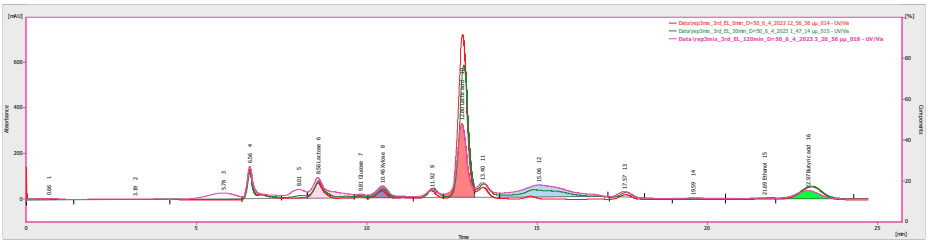

Fig. 5. HPLC chromatogram for the ethyl lactate production reaction with a catalyst at a weight fraction w_{cat}=0.3 and initial lactic acid concentration of 224.9 g/L.

4 Conclusions

This study delved deeply into the production of lactic acid and ethyl lactate from biowaste generated in a baby food industry, yielding highly significant findings. Specifically, it was demonstrated that lactic acid production from organic waste is technically feasible and offers high yields; therefore, the crucial aspect is its recovery.

Additionally, it is noteworthy to mention that the cultivation of microorganisms is an essential process for lactic acid production, as well as the medium in which they thrive, MRS. The selection of this medium proved successful, as it yielded good results.

Furthermore, the production of lactic acid was deemed feasible, albeit requiring further research. Experiments were conducted to explore various parameters aiming to identify the optimal process, while maintaining constant load quantity, temperature, volume, and stirring rate. It was revealed that experiments with 5 μg/g lactose exhibited maximum production (15g/L) within a shorter timeframe (168h) with a 48% yield. Therefore, employing a reduced lactase loading is considered optimal for lactic acid production, resulting in the highest productivity. Additionally, the influence of pre-cultivation time of microorganisms before their addition to the solution resulted in similar lactic acid concentration outcomes. However, experiments with a 48h cultivation period showed maximum production (23g/L) within a shorter timeframe (122h) with an 88% yield. Furthermore, it was observed that the lactic acid concentration is highly influenced by microorganism loading. Experiments were conducted with loads of 5% and 10%, showing noticeable differences in experiments with higher loads. Last but not least, during repeated fermentation, the alternate addition of enzymes in each cycle significantly contributed to lactic acid production, notably increasing both the yield and productivity (24.82 g/L is obtained in the 4th run at 48 h). Conversely, continuous enzyme addition, appears to impede the process, correspondingly reducing productivity (20.64 g/L in the 3rd run at 48 h).

On the other hand, the bioethanol and lactic acid fermentation broth successfully yielded ethyl lactate production, reaching a maximum lactic acid conversion of 59.9% at 255 minutes. The average lactic acid conversion stood at 56.2 ± 3.2% within a time frame of 225 ± 35 minutes. The esterification reaction was significantly impacted by the water content in the reaction mixture. Consequently, the lactic acid conversion increased with a higher grade of reactants, highlighting a positive correlation.

Generally, the development of biodegradable solvents is poised to revolutionize industry, hence bolstering research in this realm is imperative. A comprehensive study was conducted on lactic acid production from infant food waste, yet the production of ethyl lactate certainly requires further investigation. It is essential to continue research in this direction aiming to optimize processes and possibly formulate an experimental protocol for biodegradable solvent production to be tested and implemented on a larger scale. Furthermore, at this stage of research, conducting a Life Cycle Assessment (LCA) of one of the biodegradable solvents would be particularly intriguing, allowing for examination of both technical and economic factors. Thus, the production of green solvents from organic waste would be even closer to industrial implementation.

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