

Electro-dialytic Extraction of Lactic acid from Fermentation Broth using wood wastes of Paper mill

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ABSTRACT: Lactic acid can be produced by fermentation and then lactic acid can even be extracted out from fermentation broth using electro-dialysis. Lactic acid can be estimated by HPLC using C18 column. About 29.11Kg & 10.633Kg of Lactic acid can be produced from ~343 Kg of wood rejects, which come from approximately 2.86 tonne of wood which is required for production of ~1 tonne of paper. Oven dried wood has a calorific value of 19 kilo joule per gram. After fermentation wood left out can be oven dried and its calorific value would be around 15 kilo joule per gram.

INTRODUCTION

Lactic Acid has wide applications like in food industries as preservative, pharmaceutical, chemical industries and cosmetics. Lactic acid act as a monomer of biodegradable poly (lactic acid) which further has many applications. PLA is well known as bio-plastic material. US FDA(Food and Drug Administration) gave a lactic acid certification of GRAS(generally recognized as safe) as its usage in food additive. Lactic acid can be produced either by chemical synthesis or by biotechnological fermentation. But lactic acid production by fermentation is far better than chemical synthesis as it has less environmental and less use of petrochemical input. Fermentation produce optically pure lactic acid either L(+)- or D(-)- which can be polymerized to highly crystalline form poly(Lactic acid) which has uses at commercial level.. Lactic acid can be produced from several inexpensive raw materials.

Microorganisms like bacteria and fungi can be used for Lactic acid poduction. Use of fungi for fermentation has several disadvantages like lower yield and production of several

byproducts like formic acid and ethanol. So to overcome aforementioned disadvantages of fungal fermentation lactic acid bacteria (LAB) are commonly used for the fermentation.

Most of bacteria used for fermentation belong to the genus *Lactobacillus*(1,2). Stanadard Microbial strain was taken for the production of lactic acid i.e. *Lactobacillus rhamonusus* (MTCC1408).

Lactic acid concentration was measured with the help of HPLC. HPLC is a high performance liquid chromatography used to separate a mixture of several components. It has many applications like blood testing, testing of drugs in urine, separating components of biological samples, synthetic chemicals from each other and production process of pharmaceutical and biological products. Chromatography is based on mass transfer involving adsorption, portioning and desorption. HPLC has a pump which passes the liquid in pressurized form and a sample mixture through a column filled with a sorbent, which leads to separation of components. HPLC column is generally have material in granular form which is made up of solid particles like silica and polymers, 2-15 micro metre in size. Components of the

sample separate on the basis of difference in their interaction with the sorbent particles.[4]

Extraction of lactic acid from broth is a challenge due to low yield and presence of multiple components of similar functional groups. Conventional methods of separation involved lengthy steps and often difficult due to low yield. So lactic acid was extracted by Electrodialysis. Ion exchange membranes helps in electromigration of ions which form the basis of working of electrodialysis. Electrodialysis has many applications:

- Production of salt and acids
- Dimineralization of sugar
- Blood purification
- Stabilization of wine etc.

Ion exchange membrane characteristics play major role in Electrodialysis like power consumption, long-term operation, no membrane fouling. So it is better to use low electrical resistance membrane to reduce power consumption.[5]

Material and Methods

Wood waste of chipper section of paper mill is the raw material and which was collected from Star Paper Mill, Saharanpur. Chipper section rejects contains the wood peelings along with smaller wood pieces. After that those peeling and pieces were grinded to make them in powder form.

Acidic hydrolysis was carried out. Grinded wood was hydrolyzed by adding 1 ml of 25% H_2SO_4 in 100 ml of wood solution. The acidified grinded wood solution was heated in a boiling water bath for 30mins.

For fermentation, pure culture of lactobacillus i.e. *Lactobacillus rhamnosus* (MTCC 1408) was collected from MTCC Chandigarh. This strain has capability to produce lactic acid from hydrolyzed wood that contain carbohydrates. For lactobacillus strains, we need specific media i.e. MRS media. Culture was maintained at the regular intervals of time. Lactic

acid was planned to produce from wood waste of paper mill.

Fermentation-

Three samples were prepared for lactic acid production that include

- 10g wood in 100 ml of distilled water,
- 5g wood + 5 g MRS in 100 ml of distilled water,
- 10g MRS in 100 ml of distilled water

All three samples were inoculated with 1 ml of lactobacillus culture broth (MTCC 1408). Then these samples were kept in fermenter / shaker at 37°C at 150 rpm for 48 hours.

After fermentation, we have to detect that particular compound produced in fermentation broth is Lactic acid or not. So, lactic acid can be detected spectrophotometrically. After filtering the broth absorbance was measured. As lactic acid gives the maximum absorbance at 570

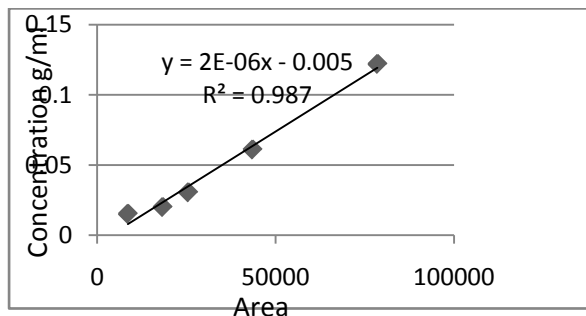
nm but in this case we didn't get absorbance exact at 570 nm but around the 570 nm, because fermentation broth has many acids apart from lactic acid like citric acid, malic acid etc.

SAMP LE	CONCENTRATI ON(g/ml)	AREA	Ret. Ti me
LA1	0.122	78533	2.38 9
LA2	0.061	43531	2.39 8
LA3	0.0305	25508	2.41 3
LA4	0.020	18339	2.41 9
LA5	0.0152	8596	2.42 0

Table1: Calibration table of lactic acid

In my project work I have already determined the optimum conditions for fermentation i.e. 28° C temperature and pH 6, we got the maximum yields at these conditions. At 28° C, we got the maximum lactic acid after 24 hours but after 48 hours lactic acid production started to decrease. All the concentrations were measured by using UV spectrophotometer but the disadvantage of using UV spectrophotometer is that we could get the concentration that may also include some quantity of other acids. So to get adequate quantity of only lactic acid. We have to use HPLC, which gives the peaks of different acids present in fermentation broth. Before the estimation of lactic acid we have prepared lactic acid calibration curve using 95% pure lactic acid further using this calibration curve lactic acid concentration in afore mentioned samples was estimated.

We are using C-18 column in HPLC system for plotting the calibration curve of lactic acid. Here we are using mobile phase i.e. 0.005 molar sulphuric acid.



Graph1: Calibration curve of lactic acid

This phase is isocratic also. The flow rate of the mobile phase was set at 0.5 ml per minute and we were getting analysis time less than 30mins with in a range of 2- 10mins.

Following three samples were fermented.

10g wood in 100 ml of distilled water,

5g wood + 5g MRS in 100 ml of distilled water,

10g MRS in 100 ml of distilled water

After fermentation those samples were filtered properly and their HPLC analysis was done to get the quantity of lactic acid produced in each samples.

After getting the quantity of lactic acid produced in each sample we had to extract out lactic acid from the mixture of organic acids those were present in fermentation broth. So for the lactic acid extraction, we use the electro dialysis method.

Electrodialysis: One stage electrodialysis with two compartment water splitting electrodialysis(WSED) was done for the lactic acid extraction using ion exchange membranes. The principle of electrodialysis is the electromigration of ions through ion exchange membrane. We are using both bipolar and anion exchange membranes alternatively.

CaCO₃ was added into fermentation broth to make the salt calcium lactate so that electrodialysis can be carried out easily. 2.922g NaCl was added in one litre of water so that ionic balance and pH could be balanced. Hydrogen and hydroxyl ions are produced from splitting of water by bipolar membrane which will be able to produce bases and acids from their corresponding salts.

After bipolar membrane, membranes of anion exchange nature help in passing of lactate ions but reject sodium ions selectively from fermentation broth. Each membrane has an area of 200 cm². Either constant voltage or constant current was set.

Hydrogen ions move more easily than lactate ions through the membrane than. In feed compartment hydrogen ions combine with lactate ions to form the neutral lactic acid. Lactate ions move mainly by diffusion. After sometime OH⁻ and H⁺ ions combine to form water molecules.

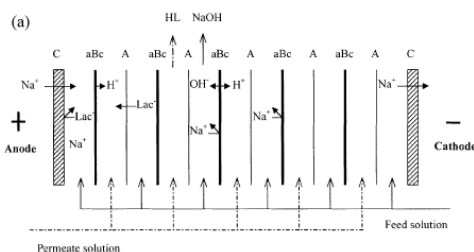


Fig6: Principle of two compartment water splitting electrodes[5]

For a given voltage the current and resistance variation were recorded using multimeter. After the extraction of lactic acid from abovementioned three samples again HPLC was done to measure the quantity of lactic acid extracted from the fermentation broth.

Still there were possibility of presence of other organic acids apart from lactic acid. In HPLC chromatogram we got many peaks which show the presence of many organic acids other than lactic acid.

But usually lactic acid showed at the retention time of between 2 and 3 min. So area corresponding to that retention time was observed and concentration of lactic acid was calculated using calibration curve.

So concentration of lactic acid in all three samples before and after electrodes was calculated and comparison was done. Electrodes was 40-50% efficient i.e only 40-50% lactic acid can be extracted out using electrodes.

So in this way lactic acid can be extracted out from the wood wastes of the paper mill. This whole procedure has dual advantages. Along with the production of lactic acid, wood can also be used for the production of steam with very slight change in calorific value of wood.

RESULTS AND DISCUSSION

Three samples were prepared for lactic acid production that include

- 10g wood in 100 ml of distilled water,
- 5g wood + 5g MRS in 100 ml of distilled water,
- 10g MRS in 100 ml of distilled water

These all samples were run at optimum conditions that were 28°C temperature and pH 6.

After filtering all samples, concentration of lactic acid was determined using HPLC. Concentration was determined before and after electrodes using calibration curve of lactic acid.

So before electrodes after filtering fermentation broths, concentration of lactic acid was calculated using HPLC.

Sample B→10g MRS in 100ml of distilled water

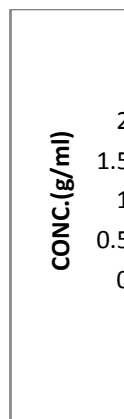
Sample D→5g MRS+ 5g wood in 100ml of distilled water

Sample E→10g wood in 100ml of distilled water

SAMPL E	CONCENTRATION(g/ml)	AREA	Ret. Time
Sample B	1.85	928207	2.746
Sample D	1.65	828248	2.310
Sample E	0.605	305088	2.875

Table2: Concentration of lactic acid before electrodes

TIME(Sec)	RESISTANCE(ohm)
0	650
33	609
840	565
1030	565
1132	565
1443	541
1616	534
1620	534
2100	534
2400	536
3606	541
4800	541



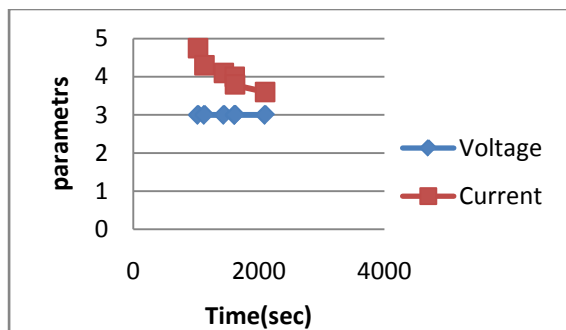
Graph h2: Conc.

Of lactic acid before electro dialysis

After observing the values of concentration of lactic acid we found out that we got maximum lactic acid in sample a that is 10g MRS in 100ml of distilled water then in 5g MRS + 5g wood in distilled water and lowest in 10g wood in 100ml of distilled water. So more MRS media in water produced more lactic acid would be there.

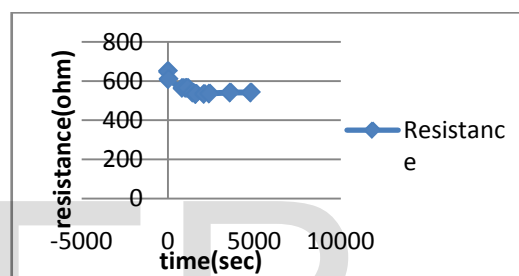
After that electro dialysis would be done and current, voltage and resistance were observed time to time.

Table3: Change in current with time during electro dialysis



Graph3: Current change with time during electro dialysis

Table4: Change in resistance with time during electro dialysis



Graph4: Resistance Vs time

Sample F→ Electridialytic extracted 10g MRS in

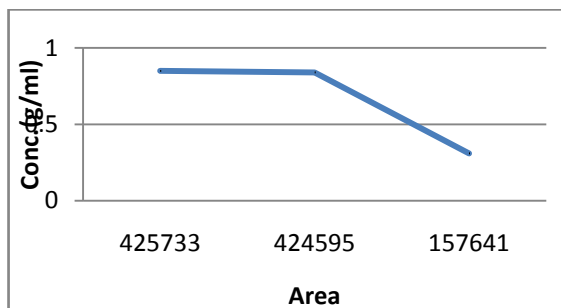
TIME(Se c)	VOLTAGE(Vol t)	CURRENT(Amper e)
0	3	5
1030	3	4.75
1132	3	4.3
1443	3	4.1
1616	3	4
1620	3	3.8
2100	3	3.6

100ml of distilled water

Sample G→ Electridialytic extracted 5g MRS+ 5g wood in 100ml of distilled water

Sample H→ Electridialytic extracted 10g wood in 100ml of distilled water

Table5: Concentration of lactic acid after electro dialysis



Graph5: Conc. of lactic acid after electro dialysis

So all these data showed that electro dialysis extracted only 45% to 50% of lactic acid. So electro dialysis was around 50% efficient. Efficiency of lactic acid depends on type of membrane used.

Further lactic acid can be polymerized into polylactic acid (PLA) and purified into pure crystalline form.

For one tonne of paper we generally require 2.86 tonne of wood where chipper section of the paper mill rejects around 343 kg of wood which they usually use for the steam production. As results have shown 10 g of oven dried wood & 5g MRS + 5g oven dried wood produce 0.605g & 1.85g of lactic acid then after electro dialysis with an efficiency of 50 % we got 0.310g & 0.85 of lactic acid. So 343 kg of wood rejects will give around 29.11 kg & 10.633 kg of lactic acid.

So, Along with the production of 1 tonne of paper we can produce around 10.7 kg of lactic acid.

If we increase the efficiency or use the better membranes in electro dialysis, we can increase the production of lactic acid around 15 kg.

Oven dried wood has a calorific value of 19 kilo joule per gram. After fermentation wood left out can be oven dried and its calorific value would be around 15 kilo joule per gram. So after analysing

SAMPL E	CONCENTRATI ON(g/ml)	AREA	Ret. Tim e
Sample F	0.85	425733	2.389
Sample G	0.84	424595	2.398
Sample H	0.31	157641	2.413

all these parameters we can say for 1 tonne of paper production along with the steam production of around 6 mega joule, we can produce lactic acid of around 291 kg & 107 kg.

CONCLUSION

It can be concluded that lactic acid can be produced from the wood wastes of the paper mill after acidic hydrolysis of wood. This wood can provide dual benefits to us that can be helpful in the production of lactic acid along with the steam production. Lactic acid is very useful byproduct with many wide applications.

After analysing all these parameters we can say for 1 tonne of paper production along with the steam production of around 6 mega joule, we can produce lactic acid of around 291 kg & 107 kg.

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