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Optimization of lactic acid production from beet molasses by Lactobacillus pentosus

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Abstract: *Lactic acid is an industrially important product with a large global market. The current study depicts that the bioproduction of lactic acid by Lactobacillus casei was optimized using 21% molasses pollutant, 5.9 pH, 33°C temperature and 52 hours of incubation period along with some other necessary growth ingredients.*

Background*: Molasses is a nicely thick as well as brown. It is converted to deep black. It is further converted to honey-like substance. It is prepared while the sugar prepared from the cane and/or the beet is finely processed. In many countries, it is then enjoyed, where it acts as a sweetener. It is the particularly used in England. Here it is called treacle. Lactic acid (2-hydroxy Propanoic Acid) is an organic compound with the formula* $CH_3.CH(OH)COOH$*. In its solid state, it is white and water-soluble. In its liquid state, it is colorless. It is produced both naturally and synthetically. With a hydroxyl group adjacent to the carboxyl group, lactic acid (2-hydroxy Propanoic Acid) is classified as an alpha-hydroxy acid.*

Materials and Methods*: Biotransformation to homolactic acid has been discussed here. It includes chemical cleaning and steam sterilization of glassware (fermentor flask, petri-dishes, platinum needle, pipettes and micro-pipettes) preparation and sterilization of different media, culture medium, inoculum medium and production medium, seeding of culture tubes, inoculation of inoculum medium and production medium preparation of buffer solution, incubation of culture tubes, inoculum medium, mutation medium.*

Results*: It is concluded that from biotechnological production of lactic acid by Lactobacillus casei is specifically increases on particular concentration of molasses. The composition of the solution is 21% (w/v) in this specific conditions. By maintaining the pH values of fermenting medium at 5.9, it is allowed to ferment for six days of the period of incubation at temperature of 30°C. It is along with other bioingredients. Here the supplements is required by Lactobacillus casei.*

Conclusion: *The current study depicts that the bioproduction of lactic acid by Lactobacillus casei was optimized using 21% molasses pollutant, 5.9 pH, 33°C temperature and 52 hours of incubation period along with some other necessary growth ingredients.*

Key Word*: lactic acid, beet molasses, Lactobacillus pentosus*

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I. Introduction

Lactic acid is an industrially important product with a large global market. Worldwide production in 2005 (exclusive of polymers) was 100,000 tons, with a 15% annual growth rate [1]. Food-related applications are the major use of lactic acid in India, accounting for approximately 85% of the commercially produced product [2]. Lactic acid is used as a buffering agent, an acidic flavoring agent, an acidulant, and a bacterial inhibitor in many processed foods.

Lactic acid (2-hydroxy Propanoic Acid) is an organic compound with the formula $CH_3.CH(OH)COOH$. In its solid state, it is white and water-soluble. In its liquid state, it is colorless. It is produced both naturally and synthetically. With a hydroxyl group adjacent to the carboxyl group, lactic acid (2-hydroxy Propanoic Acid) is classified as an alpha-hydroxy acid.

The process of lactic acid (2-hydroxy Propanoic Acid) fermentation is simple and requires little equipment. Fermentation is an anaerobic process that can proceed in any sized noncorrosive container provided it is sealed and vented for carbon dioxide release. Fermentation products can be stored until they are transported to a disposal site. For millennia, people have used lactic acid (2-hydroxy Propanoic Acid) fermentation, which is a natural process, to preserve food and feeds Fermentation is an anaerobic process in which lactic acid (2-hydroxy Propanoic Acid) bacteria transform sugar into lactic acid (2-hydroxy Propanoic Acid). Lactic acid (2-hydroxy Propanoic Acid) is a natural, low-pH, effective preservative.

Lactobacilli are potent producers of wide range of antagonistic primary and secondary metabolites such as, organic acids, diacetyl (flavouring agent), bacteriocins (nisin) and antibiotics (Ross et al'. 2002). Todorov et al. (2007) reported that Lactobacilli are also in demand for probiotic properties, as they play an important role in stabilizing the intestinal microflora by checking the colonization of pathogenic microorganisms. Lactobacillus sp. synthesize lactic acid (2-hydroxy Propanoic Acid) as their major product, that finds application in food. pharmaceutical and cosmetic industries and has various other industrial applications such as feedstock in preparation of different chemicals (acrylic acid, propylene glycol, acetaldehyde and 2,3-pentadiene), in adhesive formulation, as detergent builders, as terminating agents in phenol formaldehyde resins. Esters of lactic acid (2-hydroxy Propanoic Acid) (stearoyl-2-lactylate, glyceryl lactopalmitate and glyceryl lactostearate etc.) are used as emulsifying agents in baking foods. It also serves as raw material for the production of poly lactic acid (2-hydroxy Propanoic Acid) (PLA) which is an ecofriendly biodegradable polymer'. Adsul et al. (2007) reported that approximately 90% of worldwide lactic acid (2-hydroxy Propanoic Acid) production is through microbial fermentation and the rest is through hydrolysis of lactonitrile. Lactic acid (2-hydroxy Propanoic Acid) production through chemical synthesis provides racemic DL lactic acid (2-hydroxy Propanoic Acid) while microbial fermentation gives stereospecific L(+), D(-) or DL lactic acids based on specific microbial strains used (Altaf et al. 2006). The separation of particular form of lactic acid isomer from the racemic mixture is difficult and involves costly chromatographic techniques, hence production of lactic acid by microbial fermentation serves as a better option Narayanan et al. (2004). Presser et al (1997) reported that organic acids such as lactic acid are inhibitory towards bacterial growth as they can chelate essential growth elements like iron, while its undissociated form is lipophilic that could enter the bacterial cell and cause greater inhibition than externally active strong mineral acid. It has been reported by Mirdamadi et al (2002) that without pH control of the fermentation broth, yield of lactic acid decreases by 30 - 50%, hence neutralizing agents such as sodium hydroxide, calcium carbonate and ammonium hydroxide are usually added, for higher yield of lactic acid. Inhibitory effect of $CaCO_2$ has also been observed on growth and production of Lactobacillus strains and pellet formation in Rhizopus oryzae. Sodium and calcium ions play biologically significant roles in prokaryotic and eukaryotic cells. It is reported by some workers that calcium is present in lower concentration than the sodium ions in the cells, and the cells keep calcium ions at low level as at higher concentrations calcium ions can bind to proteins and alter their enzymatic properties.

II. Material and Methods

Biotransformation to homolactic acid has been discussed here. It includes chemical cleaning and steam sterilization of glassware (fermentor flask, petri-dishes, platinum needle, pipettes and micro-pipettes) preparation and sterilization of different media, culture medium, inoculum medium and production medium, seeding of culture tubes, inoculation of inoculum medium and production medium preparation of buffer solution, incubation of culture tubes, inoculum medium, mutation medium. Colorimetric determination of homolactic acid formed and molasses (substrate) left unfermented during the course of present investigation Search for Fermentation biotransformation of molasses pollutant to homolactic acid has also been studied. The medium for the bioproduction of lactic acid by using Lactobacillus Pentosus (3122) has the following composition:

Malt-Extract	:	0.25%
Yeast-Extract	:	0.25%
Peptone	:	0.25%
Distilled water	:	To make up 100 ml

The pH of the medium was adjusted and was maintained by adding requisite amount of lactic acid solution.

III. Result and Discussion

Molasses is a nicely thick as well as brown. It is converted to deep black. It is further converted to honey-like substance. It is prepared while the sugar prepared from the cane and/or the beet is finely processed. In many countries, it is then enjoyed, where it acts as a sweetener. It is the particularly used in England. Here it is called treacle.

For many decades, the molasses and the sulphur, and/or the treacle and the brimstone were actually thought containing some healthful benefits. There for small children dosed with the product. It is found that the molasses has some different history, which mostly at the time during prohibition in the US. For the manufacture of beverage like rum, it has the primary base.

With the bootlegging industry, the molasses importation can have some synonymous and therefore with organized crime. Nowadays, the uses of the molasses are quite benign. In baking, it is many times used primarily.

Molasses is vital and important ingredient in the cultural event. Thanksgiving holiday is one of them. In England, the treacle tan, which is many times made with molasses. The molasses is also enjoyed even on Porridge, where it acts as a sweetener. Molasses has many nice ingredients which has good nutrition. It acts like a healthy food. It has a great value then the docs white or the brown sugar.

The process of extracting molasses is very clear and straight forwards. It has some amount of sulphur, which results in fortification of calcium, iron, and magnesium. The properties of the molasses are very much presented by Some natural health food expert. They tell about the ailments of the stomach. The properties mostly related to the sulphur. This is the reason many brands of the molasses are flourished in the market, which are sulphur-free. Therefore, these products are found in chain grocery stores and natural food. Molasses is also having similar number of calories as that of sugar. It is nearly 16 calories per teaspoon (5 ml). Half the sucrose is as sugar in these cases. These molasses are high in iron and similarly high in calcium. Therefore, iron is prevented to be absorbed by the body. This is the reason the benefits can be very much discussed as a mineral supplement.

Molasses is a viscous by-product during the production procedure of the grapes, the sugar cane, or the sugar beets into the sugar. The word molasses is rooted from a famous Portuguese word, which is melap. It is a superlative form, which originates from a Latin (and Portuguese) word. It is honey. The quality of molasses very much depends on the procedure it is prepared. The process on which it depends is the way of preparing the sugar cane or the sugar beet, further, the process of sugar extracted. It also depends on the method of extraction.

In some areas/places of the United States, Sweet sorghum syrup is known as the molasses. But it is not true molasses as per current understanding. The molasses further utilized in production of lactic acid. Molasses are further the by - products of the processing industry of the sugarcane. It consists of 52% total sugars, which is then calculates as sucrose. It contained 30 % of sucrose, and 22% of invert sugars. It is popular as the black-strap molasses. This molasses acts as a component of the fermentation medium. It is considered as to contain the approximately 50% fermentable sugars. Molasses is used here in this study due to its various properties. The main property is that it is rich in sugar contents. Further it is also economical. Therefore, it is used as a carbohydrate source for the biotechnological production of the lactic acid by Lactobacillus casei.

Table-3 as well a 6 depicts the data recoded by changing the pH of the medium and the effect has been seen on biotechnological production of the fermentation of lactic acid by Lactobacillus casei. There could be two very diverse situation one could be acidic and another could be basic. These two conditions are described on the basis of its chemical property. On mixing these acids and bases, sometimes it can cancel out and/or neutralize. This flash out the effects of both acid and bases. This means it brings their extreme effects closure to each other. This is the condition when a substance cannot be either acidic or basic and it is neutral. It roughly measures the acidity. This character of the solution is measured by pH. The p stands for potent. This stand for the potential while the H stands for Hydrogen. Combinedly the meaning of pH is potential of hydrogen. This scale shows that how much acidic or basic the solution or medium is.

The scale of pH ranges from 0 to 14. Here pH value 7 means that the solution is neutral. Further a pH less than 7 means that the solution is acidic. Oppositely pH greater than 7 means that the solution is basic.

The pH scale is a logarithmic scale and therefore nearby values on pH scale is 10 times of the next hinger values. The example is as follows. pH value is 3. It is the ten times acidic than pH scale having value of 4. Further it is the hundred times (that is 10 times 10). It is the more acidic than pH 6.0. pH value is below 7. It is the ten times more acidic than the next higher value. The same is holding true for pH values above 7. Each of which is the ten times more alkaline. It is another way to say basic. It is than the next lower in the whole value. The example is as follows. If the pH value is 10. It is the ten times more alkaline than pH 9. It is100 times, which means 10 times 10 times. It is more alkaline than pH value having eight. The Pure water is then neutral. The acidic or basic depends upon the chemicals, when it is mixed with water. Either acidic or basic, it depends upon the mixture how it is prepared. Vinegar and lemon juice are the examples of acidic substances. Basic substances are in the milk of magnesia, lye, and ammonia.

It has been found that by increasing of the value of pH, the biotechnological production of the lactic acid by the Lactobacillus casei. It increases towards the acidic side of neutrality. The acidic medium solution further inhibits the fermentative production of the lactic acid. On increasing the pH values from 5.0 to 6.2, it has been found that, the lactic acid production also advances, i.e., 4.315 g/l00 ml to 8.448 g/l00 ml. It is for the biotechnological production of lactic acid by using Lactobacillus casei. The biotechnological Production of the lactic acid does not proceed by Lactobacillus casei in the condition of strong acidic and neutral pH medium smoothly. At the pH value of 5.9, the suitable condition is reached for biotechnological production of lactic acid by using the Lactobacillus casei. Thus for biotechnological production of the lactic acid (2-hydroxy Propanoic Acid), all the experimental condition is conducted by the Lactobacillus casei. It is then maintained at optimum pH value of 5.9.

The results of colorimetric analysis are given in the Table 1-6. The value reported are mean of three trials in each case.

Table 1: Study of the effect of the different carbohydrates on the biotechnological production of the lactic acid (2-hydroxy Propanoic Acid) by the lactobacillus casei

S. No.	Substrates taken for fermentation	Yield of lactic acid* in ml /100 ml	Sugar left unfermented g/100 ml.
1	Arabinose	1.396	-
2	Rhamnose	0.515	-
3	Xylose	0.479	-
4	Glucose	7.869	-
5	Fructose	5.986	-
6	Galactose	5.128	-
7	Sorbose	0.715	-
8	Lactose	4.958	-
9	Sucrose	7.953	-
10	Maltose	1.951	-
11	Starch	0.365	-
12	Inuline	0.647	-
13	Dextrine	0.547	-
14	Raffinose	1.541	-
15	Mannitol	0.154	-
16	Molasses pollutant***	7.345	2.92

*Each value represents mean of thrice observation;
**As raw material the molasses was used due to economic cost.

Table 2: Study of the major effects of the concentration and the pH on the molasses on the biotechnological production of the lactic acid (2-hydroxy Propanoic Acid) by the Lactobacillus casei in Six days of the incubation period at the pH of 6.2 and the temperature of 30°C

S. No. of sets of fermentor flasks	% concentration of molasses in gm (W/V)	Yield of lactic acid in g /100 ml
1	5%	1.45
2	10%	1.59
3	12%	1.97
4	16%	2.35
5	18%	3.69
6	20%	4.86
7	21%**	6.01***
8	25%	5.15
9	30%	4.123
10	35%	1.95

*Each value represents the mean of the three observations.
**Optimum concentration of the molasses
***Optimum yield of the lactic acid (2-hydroxy Propanoic Acid)
**** the insignificant value

Table 3: Study of the major effect of the different pH on the biotechnological production of the lactic acid by the Lactobacillus casei from the molasses (21%) in Six days of the incubation period at the temperature of 30°C

S. No. of sets of fermentor flasks	pH	Yield of lactic acid in g /100 ml	Molasses left * unfermented in g/100 ml.
1	4	2.12	–
2	4.5	2.56	–

3	5	2.9	–
4	5.2	3.45	–
5	5.7	4.89	–
6	5.9**	5.45***	3.152
7	6.2	4.56	–
8	6.5	3.95	–
9	6.8	3.2	–
10	7	2.45	–

*Each value represents mean of three observations.
**the Optimum pH values
** the optimum yield of the lactic acid.
**** the insignificant yield of the lactic acid.

Table 4: Study of the effect of the different temperature on the biotechnological production of the lactic acid by the Lactobacillus casei from (molasses 21%) in the six days of the incubation period at pH 5.9.

S. No. of sets of fermentor flasks	Temperature in °C	Yield of lactic acid * in ml /100 ml	Molasses left * unfermented in g/100 ml.
1	12	2.77	–
2	15	3.45	–
3	22	3.87	–
4	30	4.76	–
5	33**	6.14***	3.451
6	35	5.24	–
7	39	4.87	–
8	41	3.75	–
9	43	3.22	–
10	50	2.78	–

*Each value represents mean of three observations.
** the Optimum temperature
*** the Optimum yield of lactic acid
**** the Insignificant yield of lactic acid.

Table -5: Study of the major effect of the different incubation period on the biotechnological production of the lactic acid by the Lactobacillus casei from (molasses 21%) at the pH of 5.9.

S.No. of sets of fermentor flasks	Incubation period in hours	Yield of lactic acid* in g /100 ml	Molasses left * unfermented in g/100 ml.
1	20	2.25	–
2	32	3.15	–
3	50	3.45	–
4	51	4.62	
5	52**	5.89***	3.458
6	53	4.58	2.648
7	56	4.12	2.458
8	59	3.55	–
9	61	3.25	–
10	63	3.12	–
11	65	2.15	–

* the Each value represents mean of the three observations.

** the Optimum Incubation periods
*** the Optimum yield of the lactic acid
**** the Insignificant yield of the lactic acid.

The influence of the temperature can be seen in the table-4, here the data shows on the biotechnological production of the lactic acid (2-hydroxy Propanoic Acid). It is by using the Lactobacillus casei. Temperature of the medium has significant effects on the living things. Therefore, it is found that enzyme-catalysed reactions are found to be very sensitive and the small changes in the temperature are monitored. The temperature is increased from the temperature from 3°C to 30°C. It has been found that the production of lactic acid (2-hydroxy Propanoic Acid) increases. It is found that at lower temperatures, i.e., 3°C, 5°C and 10°C. The yield of lactic acid (2-hydroxy Propanoic Acid) is the yield is not very promising. Further the yield of the lactic acid (2-hydroxy Propanoic Acid) increases on increasing the temperature of the medium. It happens after 35°C and many other values above this value. The 33°C is the most significant temperature. It is the temperature where the maximum Production of lactic acid (2-hydroxy Propanoic Acid), i.e., 6.14 g/l00 ml by using the Lactobacillus casei. Further, therefore, 3.451 g/100 ml at the temperature, i.e., 33°C is selected and hence it is maintained throughout the experiments described in the thesis.

In the table-5, the data recorded reveals that the influence on biotechnological production of lactic acid (2-hydroxy Propanoic Acid) by Lactobacillus casei by various different incubation period. It has been found that conversion of molasses to lactic acid (2-hydroxy Propanoic Acid) increases. It is increasing by changing the incubation period from 1 to 6 days. The value normally falls. With the yield of lactic acid (2-hydroxy Propanoic Acid) and in 6 days, it is then found further that usually the consumption of the molasses is found, it is 3.451g/l00 ml of lactic acid (2-hydroxy Propanoic Acid), which is obtained after the production. With the increasing value of the incubation period, there is no further increase is there in the yield of the lactic acid (2-hydroxy Propanoic Acid) is found.

It is further concluded that from biotechnological production of lactic acid by Lactobacillus casei is specifically increases on particular concentration of molasses. The composition of the solution is 21% (w/v) in this specific conditions. By maintaining the pH values of fermenting medium at 5.9, it is allowed to ferment for six days of the period of incubation at temperature of 30°C. It is along with other bioingredients. Here the supplements is required by Lactobacillus casei.

IV. Conclusion

Molasses is a nicely thick as well as brown. It is converted to deep black. It is further converted to honey-like substance. It is prepared while the sugar prepared from the cane and/or the beet is finely processed. The current study depicts thatThus, bioproductionof lactic acid by Lactobacillus casei was optimized using 21% molasses pollutant, 5.9 pH, 33°C temperature and 52 hours of incubation period along with some other necessary growth ingredients.

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