Optimization of Fermentation Temperature and PH for Improved Lactic Acid Yield with *Rhizopus Oryzae*, Using Banana Peels as Substrate.

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Abstract

Lactic acid has a long history of uses for fermentation and preservation of human foodstuffs, and it is classified as GRAS (Generally Recognized as Safe) for use as a food additive. Lactic acid can be produced by either microbial fermentation or chemical synthesis. This study optimized some fermentation conditions for a better lactic acid yield, using indigenous isolate of Rhizopus oryzae. Submerged fermentation was used for lactic acid production by R. oryzae in Erlenmeyer flask containing enriched broth medium. The amount of lactic acid yield in the medium was determined by the standard titration procedure for total titratable acidity (TTA). The initial lactic acid amount in the fermentation medium was 3.70g/l, and there was progressive increase in lactic acid production which was observed from day 2 to day 4 for the different pH values used. The highest amount of lactic acid (4.44 mg/ml) was produced on the fourth day with pH of 5.5. Lactic acid highest yield was also observed with temperature of 30^0c, on the fourth day of fermentation. Temperature and pH are vital growth conditions/factors that influenced the production of lactic acid.

Keywords: Lactic acid, Optimization, Submerged fermentation, Inoculum, Rhizopus oryzae

Introduction

Lactic acid ($C_3H_6O_3$) is a valuable organic acid because of its wide use in food and food-related product. Lactic acid studies nowadays become popular with go green campaign, due to its ability to form poly lactic acid (PLA) which can be used as a material for manufacturing biodegradable polymer, including biodegradable plastic to solve one of global warming causes (Titik*et al*., 2014. Lactic acid can be produced by chemical synthesis or fermentation and 95% of industrial production of LA is obtained by fermentation pathway since it generates optically pure lactate, and also, byproducts from this process do not pollute the environment (Krishna *et al*., 2018). However, this natural way of producing LA has a real production cost problem caused by the use of expensive substrates, especially carbon sources (glucose, sucrose, lactose, and maltose) and also nitrogen sources (yeast extract, ammonium sulphate, and peptone). Furthermore, the availability of LA and also its accessibility for the various applications in the different aforementioned industries would thus be limited. It is therefore essential to search for alternatives to these expensive substrates (Coelho *et al*., 2011).

Chemical lactic acid production results only in a mixture of the two isomers, while fermentation process can yield the either form alone, or a mixture in different proportions of the two isomers, depending on the microorganism, substrate and growth conditions (Bogaert and Coszach, 2000). Indeed, the production medium plays an important part in the cost of producing biomass, metabolites, and therefore Lactic acid (Ngouénam*et al*., 2021). Several scientific studies have focused on the use of by-products from food industry as a carbon source (molasses, fruit by-products, whey, cassava bagasse, starch, sweet sorghum juice, and

lignocellulose biomass) and also as a nitrogen source (corn steep liquor, malt buds, red lentil, and baker's yeast cells) for the Lactic acid production (Ge *et al*., 2014).

Most lactic acid production through fermentation were carried out with Lactic Acid Bacteria (LAB) although, some filamentous fungi may also be used to produce lactic acid (Tay and Yang, 2002). It is therefore imperative to optimize some of the production parameters for maximum lactic acid yield, using indigenous fungal isolates.

Materials and Methods

Sample Collection and Preparation

Five hundred grams (500 g) of banana peel from fresh banana obtained from Birnin-Kebbi central market was dried at room temperature to a constant weight. The peel was grinded using the mortar and pestle into fine particles which later serves for fungi isolation and also as substrate for fermentation.

Isolation and Identification of *R. oryzae*

Potato Dextrose Agar (PDA) was used in isolating *R. oryzae*. Prepared banana peel was used to isolate the indigenous *R. oryzae*. Identification at species level was done according to Titik *et al*. (2014). The spores were harvested and suspended in 1 % Tween 80 before centrifuging at 4000 rpm for 15 minutes. The spores were estimated using a haemocytometer and observed before being transferred into the fermentation's flask (Zain *et al*., 2021).

Screening of Isolates and Media Preparation

Bromocresol Purple (BCP) agar medium was used for screening *R. oryzae* isolates. The screening was carried out at a temperature of 30^0C for 24 hours. BCP agar contains BCP indicator which shows yellow color in pH of 5.2. A yellow zone around the colony indicates a potential for lactic acid production and screening was carried out on this basis. *Rhizopus oryzae* isolates with the highest potential was employed in the fermentation process (Titik *et al*., 2014). The enriched broth medium for the fermentation was prepared as follows: 2.0 g/L $(NH_4)_2SO_4$, 0.25 g/L MgSO4. $7H_2O$, 0.04 g/L $ZnSO_4.7H_2O$, 0.2 g/L KH_2PO_4, 10 g/L $CaCO_3$, 0.03 g/L $MnSO_4.H_2O$, and 0.03 g/L $FeSO_4.H_2O$ were weighed and mixed with 1 L distilled water (Fu *et al*., 2016).

Fermentation Process

The submerged fermentation process for lactic acid production by *R. oryzae* was done by adding 2 g of banana peel powder into the Erlenmeyer flask of the enriched broth medium as prepared above. A spore solution of 4×10^4 spores/ml was inoculated into 250 ml Erlenmeyer flask containing 100 ml of the culture medium. The culture temperatures were (27^0C, 30^0C, and 35^0C), while pH was maintained at 4.5, 5.5 and 6.5. Agitation in reciprocal shaker was set at 130 strokes per min (spm) for 6 days (Kumar and Shivakumar, 2014).

For the estimation, sample was filtered using filter paper to separate medium from fungal biomass. pH meter (model Hellog Ph-3C) was calibrated with buffer solutions until values become stable and used for measuring the pH value of the medium in each Erlenmeyer flask, for 6days. The amount of lactic acid produced in the medium was determined daily for 6 days by the standard titration procedure for total titratable acidity (TTA). Each ml of 0.1M NaOH is equivalent to 90.08mg of lactic acid (Ramadzanti, 2006).

Results and Discussion

Table 1. Fungal Isolates from Banana Peel

Macroscopy	Microscopy	Isolate
White and became grayish brown with time	Non septate hyphae	*Mucor*.Spp.
Yellowish green	Septate hyphae appear at the end of the vesicle with conidia at the tip of philanders	*Aspergillus flavus*
Greyish-white colour	Non septate. Hypha becomes yellow. Visible sporangia and sporangiospores.	*Rhizopus oryzae*
Black	Blackish brown philanders with large conidiophores	*Aspergillus niger*
White	Brown-like conidiophores with whitish septate hyphae	*Penicillum* spp.

Source: Researchers' Laboratory Survey, 2024

The result presented in table 1 shows the presence of different fungi in the banana peel sample, and identifies banana peel as a good substrate for fungal growth and proliferation. This result agrees with the findings of Yun *et al.* (2003) who reported that microorganisms that can produce lactic acid can be divided into two groups: bacteria and fungi, and that most investigations of lactic acid production were carried out with lactic acid bacteria (LAB), filamentous fungi, such as *Rhizopus*, which utilizes glucose aerobically to produce lactic acid. Furthermore, in this study, agricultural waste (banana peel) was discovered as substrate for isolating citric acid producing fungi. Also reported were the utilization of corncob, waste paper and wood, and the production of lactic acid from agricultural residues such as alfalfa fiber, wheat bran, banana peel, corn stover, and wheat straw.

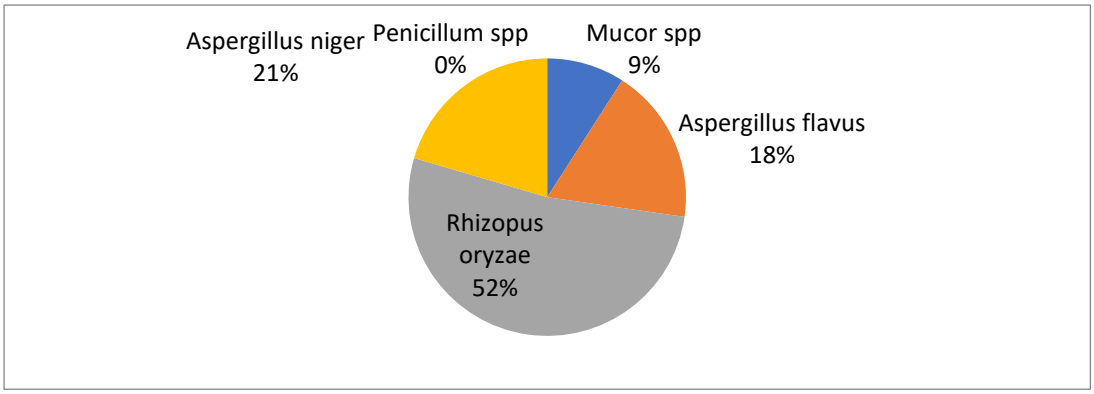


Figure 1. Lactic Acid Producing Ability of Fungal Isolates

The result of lactic acid-producing ability of fungal isolates was presented in Figure 1. *Rhizopus oryzae* showed a 2.3mm zone; a clear indication of its ability to produce lactic acid more than the other isolates. This is in Accord with Park *et al.* (2008) which reported that lactic acid production was enhanced in a culture of *R. oryzae*, by the induction of mycelial floc morphology. Furthermore, *R. oryzae* grows better under limited nitrogen condition, if compared to LAB and that fermentation using *R. oryzae* yields valuable by-product, fungal

biomass, which can be used in biosorption processes for purification of contaminated effluent and as additive in animal feeds to improve the feed quality. Comparing in downstream process, fermentation using *R. oryzae* has cheaper cost because of its easy separation between fungal biomass and fermentation broth.

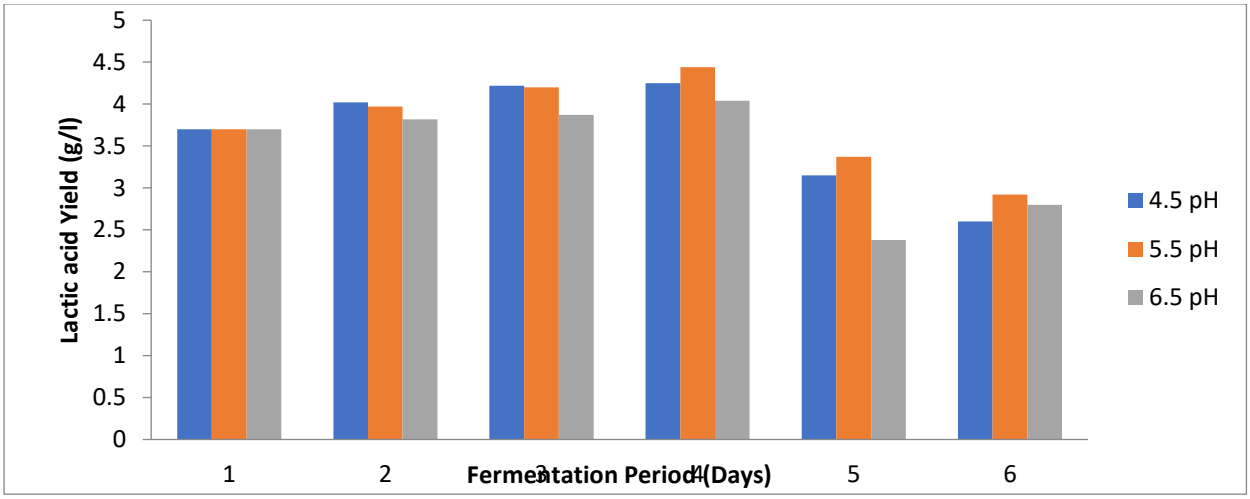


Figure 2. Result of Lactic Acid Yield with Varying pH

The result of lactic acid yield with varying pH is presented in Figure 2. The result depicted the highest amount of lactic acid (4.44 mg/ml) was produced on the fourth day with pH of 5.5, which shows this pH value to be the optimum for maximum production of lactic acid amongst the three (3) pH used. Again, highest lactic acid concentration in glucose medium was reached in 4 days fermentation (11.61 g/l), and above day 4, it then decreased at day 5 (7.83 g/l). This could be attributed to inhibition activity by some conditions; that at pH near or below pK of lactic acid (pK=3.8), inhibition on lactic acid production may occur (Skory, 2003).

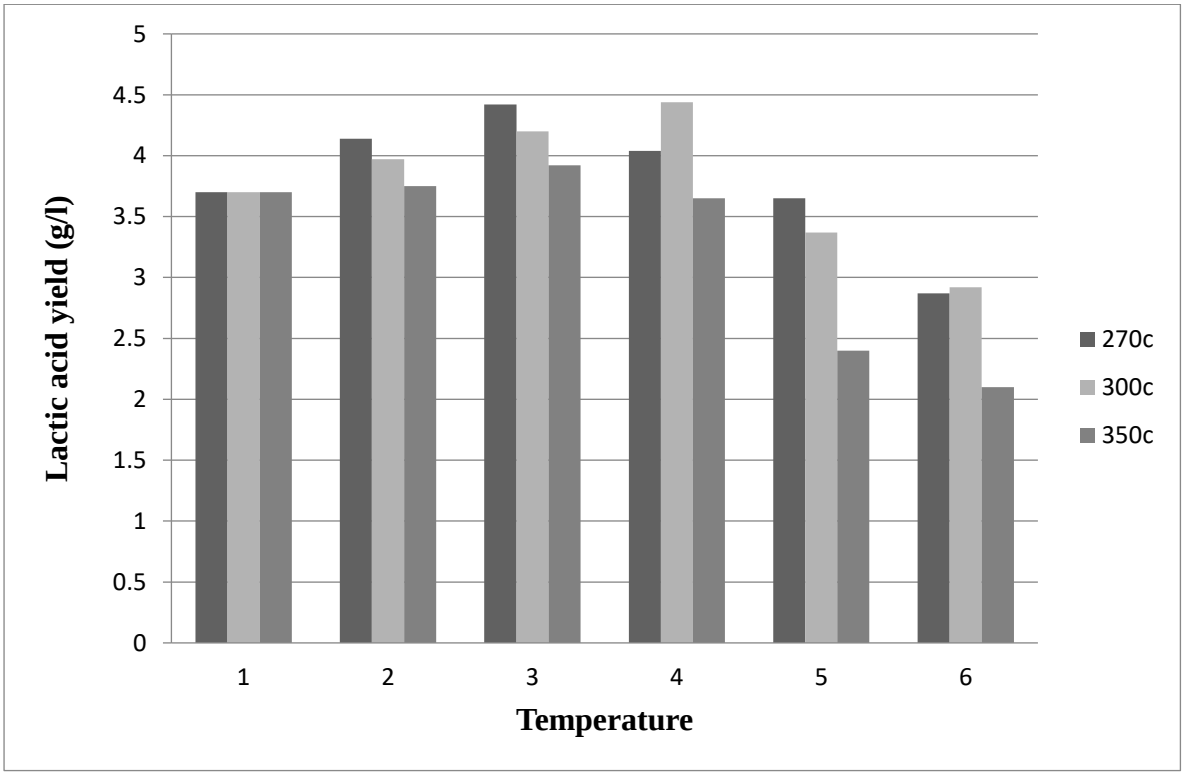


Figure 3. Result of Lactic Acid Yield with Varying Temperature

The result of lactic acid yield with varying temperature is presented in Figure 3. Lactic acid highest yield (4.44 mg/ml) was observed with temperature of 30^0c, on the fourth day of fermentation. Therefore, the temperature of 30^0c indicates the optimum temperature for maximum lactic acid yield, amongst the three (3) temperature ranges used. This result is in agreement with the results of Hofvendahl and Hahn-Hagerdal (2000) which reported that temperature and pH are also important factors influencing microbial growth and lactic acid production.

Conclusion

In a study to optimize some growth conditions for an increased lactic acid yield, different fungi were isolated from dried banana peel preparation, *Rhizopusoryzae* showed high potentials for lactic acid production after screening the isolates. The initial lactic acid amount in the fermentation medium was 3.70g/l, which progressively increased with the submerged fermentation as observed from day 2 to day 4 for the three (3) pH values used. The highest amount of lactic acid (4.44 mg/ml) was produced on the fourth day with pH of 5.5, which shows this pH value to be optimum for maximum production of lactic acid. Lactic acid highest yield (4.44 mg/ml) was also observed with temperature of 30^0c, on the fourth day of fermentation. Temperature and pH are vital growth conditions/factors that influence the production of lactic acid and, using their optimum values may yield maximum amount of lactic acid, provided other growth conditions are favourable for the production. The result has shown that banana peels are good substrate for *Rhizopusoryzae* in the production of lactic acid and biotechnology could be applied to obtain a purified product since the result is reproducible by the method used.

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