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To cite this article: Tri Candra Setiawati *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **347** 012100

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## The activity of Potassium and Phosphate Solubilizing bacteria from sugarcane rhizosphere on Some Bagasse Condition media inoculated by Lignocellulolytic bacteria

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**Abstract.** The main aim of this research was to study the activities of phosphate solubilizing (PSB) and potassium solubilizing (KSB) bacteria and lignocellulolytic on some conditions of bagasse media. Six conditions of bagasse were combined with lignocellulolytic bacteria as carrier media for PSB and KSB. Bacteria activity under observation included respiration, concentration of P-total, K-total, N-total, organic carbon, the change of C/N ratio, and the change in cellulose and lignin concentration. The research results corroborated that respiration of microbial increase from D+7 (seventh days) to D+28 (twenty-eighth days) on all condition of bagasse media with maximum respiration reaching 11.14 mg CO<sub>2</sub>/kg/day; the population of PSB and KSB are 97.67.107 cfu.g<sup>-1</sup> and 64.17. 107 cfu.g<sup>-1</sup>; the concentration of K-total and P-total, N-total, C-organic and C/N ratio decreased on all treatments media during incubation respectively.

### 1. Introduction

One of the largest agro-industrial by-products in East Java, Indonesia, is sugarcane bagasse, a fibrous residue of sugarcane stalks left over after the crushing and extraction of sugarcane juice. The potential of bagasse in Indonesia, according to the sugar plantations of Indonesia Research Center (P3GI) in 2012, is quite substantial. The average composition of a by-product of sugar industry in Indonesia consists of 52.9% liquid waste, 3.5 % blotong, 32.0% bagasse, 4.5% molasses and 7.05 % sugar and 0.1 % slag. Bagasse is one kind of lignocellulolytic biomass (LCB) which offers added values. Bagasse composition depends on several factors, including variety, growing media condition as well as environment, plant age, and harvesting condition. Some reports have described the conversion of bagasse into value-added compost that is the potential to improve the productivity of crops or as functional microorganism carrier to surmount the problem of environmental pollution.

Agricultural crop wastes, such as bagasse and rice straw, mainly consist of cellulose and hemicellulose, while the rest comprises of lignin, nitrogenous compounds, and ash. The basic compositions of bagasse, as founding in some previous studies, cover 26-47% cellulose, 19-33% hemicellulose, 14-23% lignin and 1-5% ash [1]. Mineral content of bagasse also supports the development of microorganisms when used as a carrier medium. The biological degradation of sugarcane bagasse results from the enzymatic hydrolysis of the glycosidic linkages from the cellulose chains and an attack on the lignin polymer. A study by Woo, et al [2] demonstrates that lignocellulolytic bacteria originating from tropical forest soil has the ability to decompose lignocellulose by using enzyme which produces all three glycoside hydrolase (GH) activities involved in complete enzymatic hydrolysis of cellulose. These general classes of enzymes are -1,4-endoglucanase (EC 3.2.1.4) which cleave internal 1-4-glycosidic linkages and are active against CMC;



cellobiohydrolase (EC 3.2.1.91) which is an exocellulase that cleaves cellobiose from the non-reducing end and is most active on crystalline cellulose; and -d-glucosidase (EC 3.2.1.21).

Potassium (K) and Phosphate (P) are essential nutrients that affect most of the biochemical and physiological processes that influence plant growth and metabolism. Both are macro elements required in large amount by plants. The potential of P and K concentrations in soil is fairly huge, but the available forms which can be absorbed by plants are generally low.

One alternative to nullifying the dependence on expensive imported fertilizers is to exploit indigenous resources, such as K-bearing minerals. The application of P-solubilizing and K-solubilizing microorganisms is a promising approach for increasing P and K availability in soils. One research in China [3] which involves bacterial strains, *B. edaphicus* NBT and its four mutants, MP<sub>s</sub>++, MP<sub>s</sub>+1, MP<sub>s</sub>+2, and MP<sub>s</sub>- (in separate tests), demonstrates that root growth and shoot growth of wheat are significantly increased and these strains as well as mutants have resulted in significantly higher N, P, and K contents of plant components.

By contrast, Han and Lee's [4] research results concerning phosphate solubilizing bacteria (PSB) *Bacillus megaterium* and potassium solubilizing bacteria (KSB) *Bacillus mucilaginosus* inoculated in nutrient limited soil planted with eggplant showed that rock P and K materials either applied individually or in combination did not significantly enhance the availability of P and K in soil. For effective application of isolate, it is imperative to apply appropriate carrier media, which are not toxic and contain sufficient organic carbon and nutrient.

Bagasse is rich in carbohydrates, mainly lignin and cellulose. The spatial structure of bagasse is formed by parallel-arranged fibres and micro pores (0.5–5 µm). It is an ideal medium for attachment bacteria and fungal hyphae.

The main aim of this research was to study the activities of lignocellulolytic, phosphate solubilizing (PSB) and potassium solubilizing (KSB) bacteria in some conditions of bagasse media through respiration as well as the change of Carbon, Nitrogen, Phosphate, and Potassium concentration.

## 2. Materials and methods

### 2.1. Bagasse Media

Bagasse used in this study was obtained from Asem Bagus sugar factory, Situbondo, Indonesia. Two conditions of bagasse are fresh bagasse (FB), produced less than 3 months, and matured bagasse (MB), which is produced more than 6 month. To accelerate decomposition of bagasse, three sizes of bagasse fraction were at play: <10 mesh (Long fiber bagasse=LFB), 10-20 mesh (short fiber bagasse=SFB), and > 20 mesh (pith bagasse=PB). The bagasse from sugar factory was cut, washed with running water for 12h, dried at room temperature and filtered to meet expected size [5]. The bagasse was sterilized three times by autoclave at temperature of 121°C for 30 minutes.

### 2.2. Isolate

#### 2.2.1. lignocellulolytic bacteria

Two isolates Lignocelluloses degrading strains I20 and 40A (a collection of microbiology laboratory, The Faculty of Mathematics and Science, University of Jember) were used to test the activity and enhance bagasse decomposition rate. After being refreshed, to be applied in bagasse media, the isolate lignocellulolytic bacteria was grown in the Nutrient broth media. The activity of lignocellulolytic bacteria under analysis was the change of lignin and cellulose concentration in bagasse media and C/N ratio of bagasse media.

Changes in cellulose content were estimated by adding 1 g decomposed matter with 15 ml of 80% acetic acid and 1.5 ml of HNO<sub>3</sub>. The mixture was then heated for 20 minutes and filtered with Whatman paper #1 and washed using hot water. This was then put into oven at 105°C overnight, weighed and heated to the muffle furnace at 550°C for 3 hours. The cellulose was calculated using the formula [6].

$$\text{Cellulose (\%)} = \frac{\text{Weight of digest matter} - \text{ash weight}}{\text{Dry weight of matter}} \times 100$$

Lignin content was estimated by means of hydrolisis on matter ( $\pm 2$  g) using 1.25% H<sub>2</sub>SO<sub>4</sub> for 2 hours and 1.25% H<sub>2</sub>SO<sub>4</sub> for 4 hours. The residue was washed using water to nullify the sulphate acid and the put into oven at 105°C for one night. The percentage of lignin was formulated using the following formula [6].

$$\text{Lignin (\%)} = \frac{\text{Lignin weight}}{\text{Material weight}} \times 100$$

### 2.2.2. *Phosphates solubilizing bacteria (PSB) and Potassium Solubilizing bacteria (KSB)*

PSB was grown in Pikovskaya media and KSB was grown in Alexandrov’s media. To test the activity of Phosphates solubilizing microbes (PSB) and Potassium Solubilizing microorganism (KSB), the research used two indigenous bacteria obtained from sugarcane rhizosphere. The experiments were set up in a complete randomized blocks design, with 3 replications and 18 combination treatments to test the activity of microbes (Table 1).

**Table 1.** Treatments of Research

Bagasse condition	Code	Isolate decomposer	Code
<i>Fresh bagasse (FB)</i>			
< 10 mesh (LFB)	B1	No decomposer	I0
10-20 mesh (SFB)	B2		
>20 mesh (PB)	B3	Strain I20	I1
<i>Matured bagasse (MB)</i>			
< 10 mesh (LFB)	B4	Strain 40A	I2
10-20 mesh (SFB)	B5		
>20 mesh (PB)	B6		

50 g sterile bagasse was inoculated by the respective isolates, with a density of approximately 1x10<sup>6</sup> cfu/g media after being propagated on nutrient broth. The first inoculation was carried out by decomposer isolate (strain I20 or 40A), followed by another inoculation using PSB and KSB. The antagonistic test results between decomposer isolates and Phosphates solubilizing microbes (PSB) and Potassium Solubilizing microorganism (KSB) isolates did not show antagonistic things. The moisture content in each treatment was adjusted to about 60-70% at the beginning of incubating, and then periodically sterile water was added during the turning of incubating up to 30 days.

### 2.3. *Analysis of samples*

Samples were analysed periodically from each treatment on days 7, 14, 21 and 28 for respiration analysis. CO<sub>2</sub> production is directly correlated with the aerobic respiration. The analysis on CO<sub>2</sub> production was done by using alkaline traps to fix CO<sub>2</sub>. The total number of bacteria were counted using the pour plate method on the selective medium at the middle and the end of the experiment. Pikovskaya’s medium (Glucose 10.0; Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> 5 g ; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5; MgSO<sub>4</sub> 7H<sub>2</sub>O 0.1g; KCl 0.2g; MnSO<sub>4</sub> 2.5mg and FeSO<sub>4</sub> 2.5mg; yeast extract 0.5g; Agar-agar 15g; pH7-7.2) for PSB and Alexandrov’s medium (composition: Glucose, 5g ; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.5 g; CaCO<sub>3</sub>, 0.1 g; FeCl<sub>3</sub>, 0.006g; Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, 2.0 g; Potassium source, 3 g, agar 15 g; yeast extract 0.15 g; D/W, 1000ml) for KSB.

While other parameter analysis on end of the experiment. Total cellulose, lignin and organic carbon content in the sample were determined by the weight loss on ignition method. Furthermore, total nitrogen was measured by the micro-Kjeldahl method, Total phosphorus was estimated by a vanadomolybdo phosphoric acid yellow color method using a Spectrophotometer, while total potassium was detected by the wet destruction method using Atomic Absorption Spectrophotometer (AAS). Ratio C: N was considered from the measured value of C and N.

### 3. Result and Discussion

#### 3.1. Microorganisms activity in bagasse media

Respiration is directly related to the metabolic activity of a microbial population. Microorganisms respire at higher rates when large amounts of bioavailable organic matter are present, while respiration rate is lower if this material is limited. Based on CO<sub>2</sub> production, microbial activity on all conditions of the bagasse media became higher as the duration of incubation increased (Figure 1). There is no significant difference in any treatment of the observed respiration until the 28<sup>th</sup> day. This is because the existing media still give appropriate conditions (moisture and temperature), carbon sources, as well as the nutrient needed. The condition is linked with the number of the respective populations of phosphate and potassium solubilizing microorganisms, as well as the existing lignocellulolytic microorganisms in the media. An increase in the activity of microorganisms from the 14<sup>th</sup> day to the next day showed good adaptability and synergisms among microorganisms. On the initial conditions to 21<sup>th</sup> day, the respiration rate in the media was a moderately low (2-8 mg of CO<sub>2</sub>. g<sup>-1</sup>. day<sup>-1</sup>) and increased to medium (9-15 mg CO<sub>2</sub>. g<sup>-1</sup>. day<sup>-1</sup>) on 28<sup>th</sup> day [7]. Microbial activity in a compost process and – by consequence – in a respirometry assay, was affected by many different factors, such as moisture content and temperature of the sample, microbial population, nutrients equilibrium, or occurrence of toxic compounds, quantity and quality of substrate [8]. The study revealed that the respiration rate up until the 28<sup>th</sup> day was very low, which was indicated by CO<sub>2</sub> production ranging from 1.94 to 11.14 mg CO<sub>2</sub>. g<sup>-1</sup>day<sup>-1</sup> or 13.58 to 77.98mg CO<sub>2</sub>.g<sup>-1</sup>. week<sup>-1</sup>.

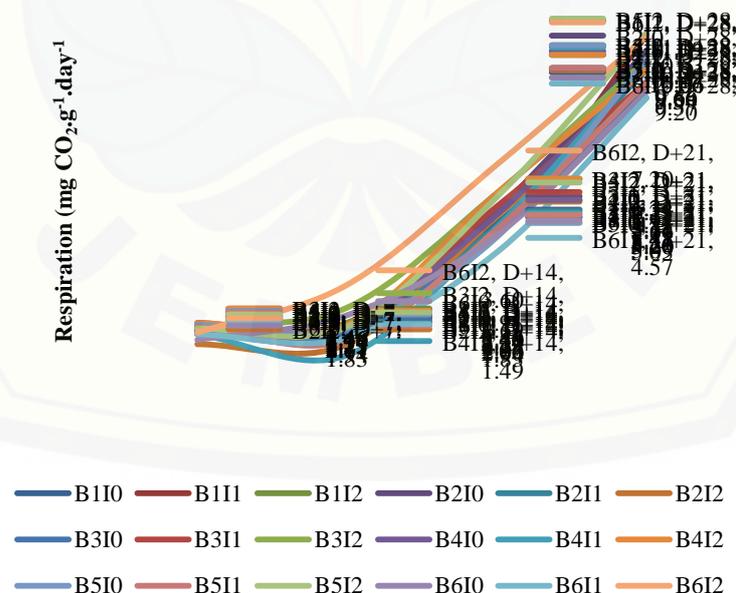


Figure 1. Respiration on bagasse media

3.2. Population and activity of microbe on bagasse media

3.2.1. Phosphate solubilising bacteria

The initial population of decomposer microorganisms in the bagasse was  $1 \times 10^6$  cfu/g media, and  $2 \times 10^6$  cfu/g media for PSB and KSB. Total population in the bagasse was  $3.0 \times 10^6$  cfu/g media. Population density increased to maximum  $9.77 \times 10^8$  cfu/g media between the 1<sup>st</sup> and 15<sup>th</sup> days of incubating. Similar to the research result, Ref. [9] demonstrate that the increasing bacterial population in the bagasse and coast-cross straw mix has increased to an average of  $7.1 \times 10^8$  CFU/g between the 2<sup>nd</sup> and 10<sup>th</sup> days of composting, after which it fell back to  $5.7 \times 10^8$  CFU/g by the 14<sup>th</sup> day.

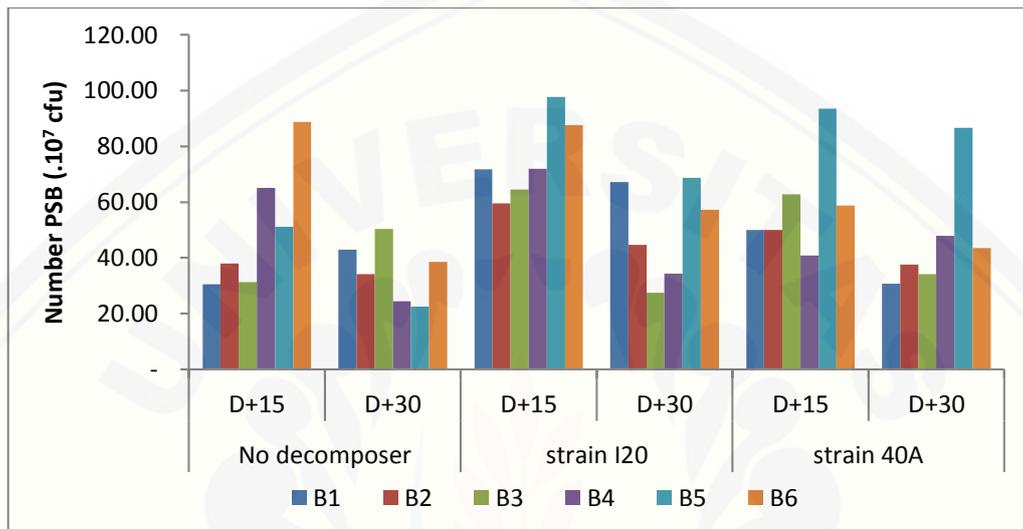


Figure 2. Population of Phosphate solubilising bacteria on bagasse media

3.2.2. Potassium Solubilising Bacteria

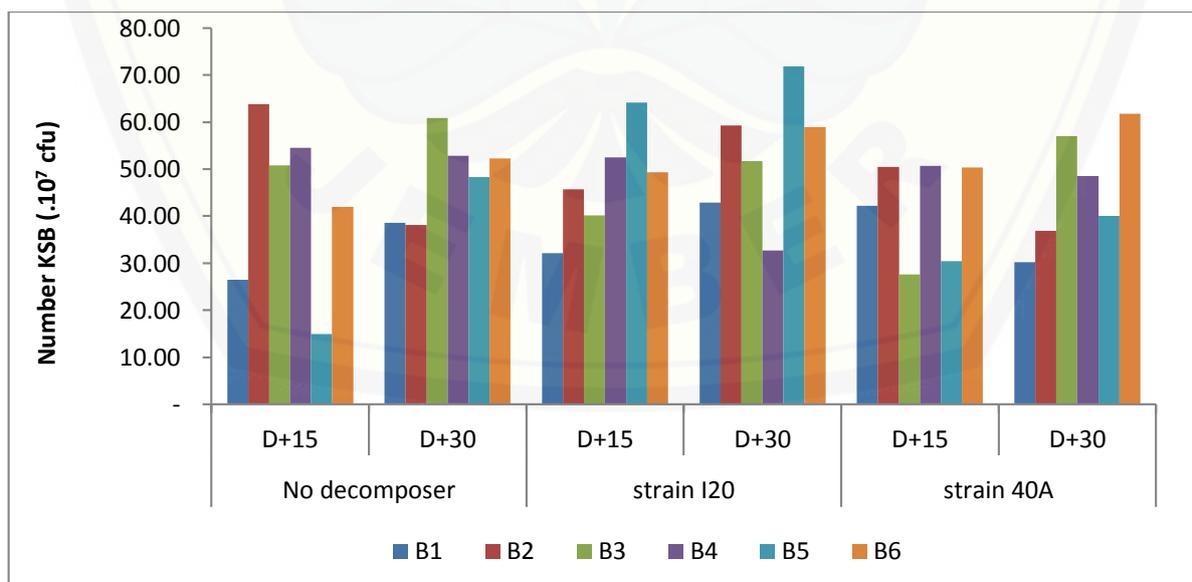


Figure 3. Population of Potassium solubilising bacteria on bagasse media

The population of PSB on 30<sup>th</sup> days of incubating (D+30), due to the inoculation by lignocelulolytic, declines, compared to 15<sup>th</sup> days (Figure 2). Only in the treatment with no decomposer is discovered the increase in number of PSB populations in NB media with size>20 mesh.

As what happened to PSB population, that of KSB also indicated significant increase (Fig. 3), compared to its initial number. The population in matured bagasse (MB) at the same size demonstrated that the cell density was greater than that of FB.

### 3.2.3. Lignocelulolytic Bacteria

Based on the research result by Ref. [10] the isolates used are strain I20 and 40A, which are negative bacteria, indicating fairly high cellulose intensity as it creates glucose reduction by 12.27 µg.ml-1 and 3.48 µg.ml-1 in CMC media. In general, the population of lignocelulolytic isolates (strains I20 and 40A) increased until 30<sup>th</sup> day compare initial density. The population counted from the 15<sup>th</sup> day to 30<sup>th</sup> day revealed that both isolates grew at higher rate in pith condition in the fresh bagasse (FB), and SFB in matured bagasse (MB) with a maximum population of each isolate reaching 24.04.107 cfu. The bagasse condition influence the development of lignocelulolytic isolates. Pith bagasse (PB) affords more spacious surface than LFB and SFB, so greater cell density is evident.

Bagasse possesses several components, which include Glucan, Xylan, Arabinan, Galactan, Acetic acid, lignin and ash, while the others are associated with cellulose [11]. Such condition will influence decomposition by lignocelulolytic bacteria. Cellulose degradation comprising of the β-D-Glucose series includes approximately 1900 glucose units (monomer). This is catalyzed by cellulase enzyme which consists of at least three enzymes viz., Endo-1, 4 glucanases, Exo-1,4 glucanases and -glucosidases [12]. The activities of lignocelulolytic microorganisms significantly decomposed lignin and cellulose in bagasse media, therefore declining lignin and cellulose at the end of incubation. Cellulose in the media with fresh bagasse (FB) decayed by 42.17%. compared to the cellulose initial content. By contrast. in the matured bagasse media (MB) cellulose declined by 14.14%. The inoculation with isolate of strains I20 decomposed cellulose more than did 40A strains, while more substantial decomposition of lignin on the fresh bagasse was evident in I20 strain, compared to the matured bagasse (MB) decomposed by strains 40A.

The percentage of cellulosa and lignin in control (No decomposer) is found higher than that of PSB, KSB, or lignocelulolytic bacteria. A study conducted by Ref. [13] indicates that the application of white-rot fungus results in the highest glucose concentration, while at the same time reducing lignin and cellulose concentration, compared to control and other treatments. This occurs due to the use of cellulose by isolate.

**Table 2.** Carbon organic (%) and n total (%) bagasse media

Bagasse condition		Isolate decomposer					
		No decomposer		Strain I20		Strain 40A	
		C-org	N-total	C-org	N-total	C-org	N-total
Fresh bagasse (FB)	LFB	48.33	1.14	53.67	1.17	58.33	1.16
	SFB	61.00	1.16	50.00	1.19	55.00	1.18
	Pith	56.33	1.14	50.00	1.16	48.00	1.15
Matured bagasse (MB)	LFB	57.33	1.17	62.67	1.19	42.00	1.19
	SFB	66.00	1.19	43.67	1.22	60.33	1.21
	Pith	48.00	1.17	43.85	1.18	54.67	1.17

The initial C-organic of each bagasse was 82.44 % (FB) and 83.43 % (MB) and both decreased by 35.06 % and 45.05% respectively after the incubation period was complete on all bagasse conditions (Table 2). The condition of bagasse (FB and MB) and size of bagasse (LFB, SFB, and Pith bagasse) posed no significant difference in organic carbon content. Inoculation with lignocelulolytic microorganisms increased decomposition of bagasse. As a result, after incubation, the content of C-

organic was reduced. The concentration N-total bagasse declined by 24.67 % in FB media and 34.62% in MB media. This caused declines in C/N ratio of bagasse.

**Table 3.** P-total (%) and K total (%) bagasse media

Bagasse condition		Isolate					
		No decomposer		Strain I20		Strain 40A	
		P-total	K-total	P-total	K-total	P-total	K-total
Fresh bagasse (FB)	LFB	0.046	0.133	0.051	0.084	0.056	0.084
	SFB	0.070	0.345	0.061	0.200	0.060	0.276
	Pith	0.054	0.064	0.057	0.127	0.055	0.151
Matured bagasse (MB)	LFB	0.062	0.289	0.057	0.212	0.057	0.227
	SFB	0.056	0.203	0.061	0.247	0.062	0.155
	Pith	0.058	0.224	0.063	0.269	0.052	0.214

The concentration of total phosphate in bagasse decreased by 40.51% in New Bagasse (NB) media and 45.79% in MB media, compared to the initial P-total concentration (Table 3). When the C:P ratio was greater than 300:1, net immobilization occurred. During immobilization, there was insufficient amount of P to sustain microorganisms. Therefore, the activity of microorganisms still utilized the mineralized phosphate from bagasse.

Intense potassium mineralization will reduce K-total concentration in bagasse (Table 3). Like PSB, KSB in its metabolism produces alkaline phosphatase and lipase enzyme. Potassium mineralization at high rate will reduce total K concentration of bagasse (Table 3).

#### 4. Conclusion

The activity of PSB and KSB on bagasse medium showed that respiration of microbial increase from D+7 (seventh days) to D+28 (twenty-eighth days) on all condition of bagasse media with maximum respiration reaching 11.14 mg CO<sub>2</sub>/kg/day; the population of PSB and KSB are 97.67.107cfu.g-1 and 64.17. 107cfu.g-1; the concentration of K-total and P-total decreased on all treatments, also N-total, C-organic and C/N ratio, media during incubation respectively.

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### **Acknowledgment**

The authors are appreciative to the Ministry of Research Technology and Higher Education for funding this PUPT Research .