PRODUCTION OF LIGNOCELLULOSIC ETHANOL FROM *Ipomoea carnea* BY BACTERIAL CELLULASE OF COW AND DEER DUNG

Submitted by

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CERTIFICATE

This is to certify that the thesis entitled " Production of lignocellulosic ethanol from *Ipomoea carnea* by bacterial cellulase of cow and deer dung" is submitted by Miss. Manisha Kumari (Roll No- 411LS2041) to this Institute in partial fulfillment of the requirement for the award of the degree of Master of Science in Department of Life Science, is a bonafied record of the work carried out under my supervision and guidance. It is further certified that no part of this thesis is submitted for the award of any degree.

Rourkela -

Date -

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Supervisor

Department of Life Science

DECLARATION

I hereby declare that the thesis entitled "**Production of lignocellulosic ethanol from *Ipomoea carnea* by bacterial cellulase of cow and deer dung**" submitted to the Department of LIFE SCIENCE, National Institute of Technology, Rourkela for the partial fulfilment of the Master of Science in Life Science is a faithful record of original research work carried out by me under the guidance and supervision of Dr. R. Jayabalan, Department of Life Science, NIT, Rourkela. No part of this thesis has been submitted by any other research persons or any students.

Date:
Place: Rourkela

Manisha Kumari

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Rourkela (Manisha Kumari)

Date:

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Abstract

Due to rapid growth in population and industrialization, worldwide ethanol demand is increasing continuously lignocellulosic biomass are most abundant and renewable sources of the world they can act as a promising source for bioethanol production. The major objective of the work was to evaluate the effect of acid and steam pretreatment On *Ipomoea carnea* for improved yield of bioethanol production by using cellulase producing bacteria isolated from Deer and Cow dung and optimization of condition required for maximum activity of cellulase enzyme. Cellulase producing bacteria isolated from Deer and Cow dung screened by congo red test. Cellulase activity was measured by DNS method. From the present study it is concluded that bacteria isolated from deer and cow dung was producing maximum amount of cellulase enzyme after 60 hours (DDB) and after 70 hours (CDB). Cellulase enzyme produced by bacteria isolated from deer and cow dung was found to have pH around 3, temperature 50°C for DDB and 60°C for CDB. Activity of Cellulase enzyme produced by bacteria isolated from deer and cow dung was found to be increased by the addition of 5 mM $MnSO_4$ (both DDB and CDB). It Possible to produce lignocellulosic bioethanol (5.52%) from *Ipomoea carnea* after steam and acid pretreatment by using Cellulase for Saccharification (72 hours) and *Saccharomyces cerevisiae* for fermentation (72 hours). Bioethanol from lignocellulosic biomass is a globally accepted alternative fuel. The production of ethanol from *Ipomoea carnea* would have the dual advantage of producing energy and serving as an effective method of weed management.

Key words: *Ipomoea carnea,* Lignocellulosic biomass, Bioethanol

1. INTRODUCTION

Bioethanol fuel has an important role to reduce global warming and to conserve fossil fuel. Ethanol Production from corn and other crops cause some problems like tightening food supplies and soaring food prizes. Hence, the production of ethanol from lignocellulose biomass such as rice straw and wood is beginning to get a lot of attention recently. Bioethanol is a type of fuel whose energy is derived from biological carbon fixation. Apart from these feed stocks, lignocellulosic biomass, which is the most abundant on earth (Purwadi et al., 2004), is an alternative feed stock for bioethanol production. This biomass including forest residues such as wood, industrial residues such as pulp and paper processing wastes are most potential feed stocks for fuel ethanol (Mielenz et al., 2011). Biofuel are graining increased public and scientific attention, driven by factors such as oil price hikes and the need for increased energy security. However, according to the European Environment Agency, biofuel address global warming concerns only in specific cases. Bioethanol is an alcohol made by fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn or sugarcane. Cellulosic biomass, derived from non-food sources, such as trees and grasses, is also being developed as a feedstock for ethanol production. The Principal fuel used as a petrol substitute for road transport vehicle is bioethanol. Bioethanol fuel is mainly produced by the sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. The main source of sugar required to produce ethanol come from fuel or energy crops. These crops are grown specifically for energy use and include corn, maize and wheat crops, waste straw, willow and popular trees, sawdust, reed canary grass, cord grasses, Jerusalem artichoke, miscanthus and sorghum plants. There is also on going research and development into the use of municipal solid wastes to produce ethanol fuel. Bioethanol is widely used in the USA and in Brazil. It is considered an alternative to petroleum and diesel and its popularity is emerging as a fuel for cars –it is particularly well established in Brazil. Ethanol is a high octane fuel and has replaced lead as an octane enhancer in petrol. By blending ethanol with gasoline we can also oxygenate the fuel mixture so it burns more completely and reduces polluting emission. Ethanol fuel blends are widely sold in United States. The most common blend is 10% ethanol and 90% petrol (E10). Vehicle engines require no modification to run on E10 and up to 85% ethanol and 15% petrol blends (E85). In 2010, worldwide biofuel production reached 105 billion litres (28 billion gallon

US), up to 17% from 2009, and biofuel provided 2.7% of the world's fuel for road transport, a contribution largely made up of ethanol and biodiesel. Global ethanol fuel production reached 86 billion litres (23 billion gallons US) in 2010, with the United States and Brazil as the world's top producers, accounting together for 90% of global production. As of 2011, mandates for blending biofuels exist in 31 countries at the national level and in 29 states or provinces. The International Energy Agency has a goal for biofuels to meet more than a quarter of world demand for transportation fuels by 2050 to reduce dependency on petroleum and coal. Biologically produced alcohols most commonly ethanol, and less commonly propanol and butanol, are produced by the action of microorganism and enzymes through the fermentation of sugar or starches(easiest), or cellulose(which is more difficult). The ethanol production method used are enzyme digestion (to release sugar from starches), fermentation of sugar, distillation and drying. The distillation process require significant energy input for heat. Energy consumption has increased steadily over the last century as the world population has grown and more countries have become industrialized. Unlike fossil fuel, ethanol is a renewable energy source produced through fermentation of sugar. Ethanol is widely used as a partial gasoline replacement in the US. Fuel ethanol that is produced from corn has been used in gasohol or oxygenated fuels since the 1980s. These gasoline fuel contain up to 10% ethanol by volume. As a result, the US transportation sector now consumes about 4540 million litres of ethanol annually, about 1% of the total consumption of gasoline (Wang et al., 1999). Biomass energy includes both traditional uses(for heating and cooking) and for modern uses (like producing steam and electricity, and liquid biofuel). Ethanol derived from biomass, one of the modern form of biomass energy, has the potential to be a sustainable transportation fuel, as well as a fuel oxygenated. That can replace gasoline. Ethanol is also a safer alternative to methyl tertiary butyl ether (MTBE), the most common additive to gasoline used to provide cleaner combustion (McCarthy and Tiemann, 1998). Using lignocellulose biomass to form bioethanol because it is most abundantly available raw material on the earth for the production of biofuel, especially bioethanol. Lignocellulose refers to plant dry matter, so called Lignocellulosic biomass. It is composed of carbohydrates polymer (cellulose, hemicellulose), and an aromatic polymer (lignin). These carbohydrates polymer contain different sugar monomers (six and five carbon sugar) and they are tightly bound to lignin. This lignocellulosic biomass can be grouped into four main categories agricultural residues. Lignocellulose biomass, in the form of wood fuel, has a long history as a source of

energy. Since the middle of the 20th century, the interest of biomass as a precursor to liquid fuel has increased. To be specific, the fermentation of lignocellulosic biomass to ethanol is an attractive route to fuel that supplements the fossil fuels. Aside from ethanol, many other lignocellulose derived fuel are of potential of interest. The enzymatic hydrolysis of cellulose is affected by several factors, i.e., degree of polymerization, degree of crystallinity, structural composition and availability of surface area, etc (Gupta et al., 2009). A Particular biomass containing more than 70% cellulose and hemicelluloses can be considered as a good candidate. The lignin content should be less than 20% (Sindhu et al., 2010). *Ipomoea carnea* fulfil all the above criteria, so it can be use as potent source for lignocellulosic biomass and bioethanol production. *Ipomoea carnea* is an important non edible lignocellulosic biomass, which grows widely throughout India. It is nonedible due to its high toxin content and has been found to be toxic to animals, several important characteristics of *Ipomoea carnea* such as easy availability, high cellulose content and no competition with the food chain makes it an ideal substrate for bioethanol production (Sharma et al., 2010).

2. Literature Review

2.1 Ethanol

Ethanol or ethyl alcohol (C_2H_5OH) is a colourless liquid, low in toxicity, biodegradable and cause little environmental pollution if split. Ethanol burns to produce carbon dioxide and water. Ethanol can be used as a fuel for vehicles in its pure form, but it is usually used as a gasoline additive to increase octane and improve vehicle emission. A big advantage of ethanol is that it has highest octane rating than ethanol-free gasoline which is available at roadside gas stations, and it is oxygenated fuel with a high octane value having a flash point of 16.6 °C. In high altitude locations, it is mandatory to mix ethanol and gasoline as a winter oxidiser to reduce atmospheric pollution. Ethanol can be used in multi field areas.

2.2 Bioethanol

Bioethanol fuel has an important role in the field of environment to migrate global warming and to conserve fossil fuel. It is an alcohol made by fermentation, of carbohydrates. It is the most widely used biofuel for transportation worldwide. Production of bioethanol from biomass is one way to reduce both consumption of crude oil and environmental pollution Cellulosic biomass derived from non food sources, such as grasses and trees, is also being developed as a feedstock for ethanol production. Bioethanol in its purest form is a colourless clear liquid with mild characteristic odour that boils at 78°C and freezes at -112°C.

2.3 Why Bioethanol?

It is well known that plant are the most common source of renewable carbon and energy on the earth and also world has been confronted with an energy crisis due to depletion of finite resources of fossil fuel, difficulties in their extraction and processing, leading to an increase of its cost which become impossible for common people to pay. As we all known fossil fuels contribute a very important role in accumulation of greenhouse gases (GHG) which can ultimately pollute the environment . Fossil fuel are being used for the production of electricity, fuel and other goods. Green House Effect has been increasing very drastically and causing disaster in the environment. The road transport network accounts for 25% of all Green House Gases emission and through the use of Bioethanol, some of these emission will be reduced as the

fuel which absorb the carbon dioxide they emit through growing. So it is essential to look forward for alternate fuel. This includes bio hydrogen, bioethanol and biodiesel. Bioethanol has a number of advantages over conventional fuel. As it comes from the renewable source of energy it serves as a best fuel with less cost. Looking for future needs bioethanol is considered as the most promising source, which completely replace fossil fuel.

2.4 The Three Generation of Bioethanol

2.4.1 First Generation of Bioethanol

It is also a conventional biofuel which are made from sugar, starch or vegetable oil. These are mainly extracted from crops like sugarcane, maize, sugar beets and molasses using conventional technology. Its growth and development is limited due to competition with food and fibre production for the use of arable land, regionally constrained market structures.

2.4.2 Second Generation bioethanol

These are also known as advanced biofuel, are fuel that can be manufactured from various types of biomass. Lignocellulosic biomass fall under this category are promising source for second generation bioethanol production (Kim et al., 2002).

2.4.3 Third Generation Bioethanol

The third generation bioethanol feed stocks, which are derived from microalgae, have emerged as one of the most promising alternative sources of lipid of their high photosynthetic efficiency to produce biomass and their higher growth rates and productivity compared to conventional crops.

2.5. Lignocellulose

Lignocellulose refers to plant dry matter i.e. biomass so called lignocellulosic biomass. It is the most abundantly available raw material on the Earth for the production of biofuel, mainly bioethanol. Lignocellulose biomass, has a long history as a source of energy. Since the middle of the 20^{th} century, the interest of biomass as a precursor to liquid fuel has increased. To be specific, the fermentation of lignocellulosic biomass to ethanol. It is an attractive route to fuels

that supplements the fossil fuel. It is composed of carbohydrates polymers i.e. cellulose, hemicellulose and aromatic polymer (lignin) Fig.1

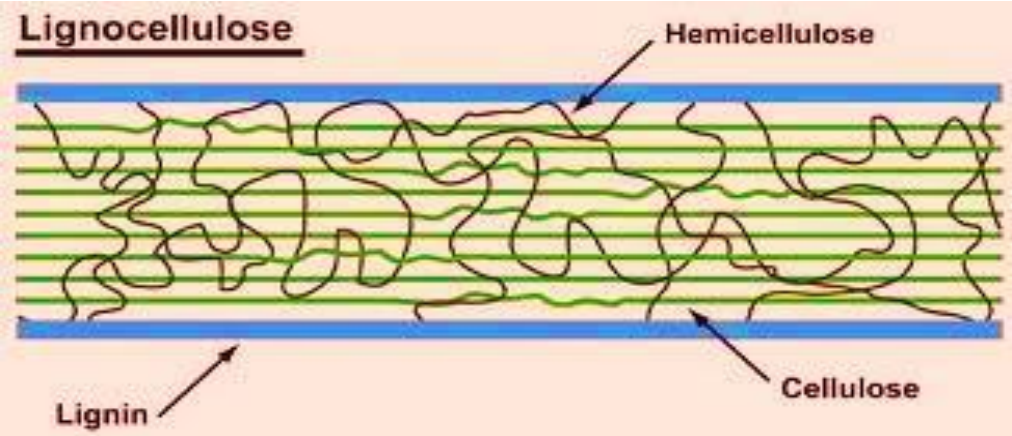

Fig.1: Lignocellulose containing cellulose, hemicellulose and lignin

These carbohydrates polymers contain different sugar monomers (six and five carbon sugar) and they are tightly bound to lignin. The complex structure of Lignocellulose in which the cellulose is surrounded by a monolayer of hemicellulose and embedded in a matrix of hemicellulose and lignin. Lignin specifically creates a barrier to enzymatic attack while the highly crystalline structure of cellulose is insoluble in water while the hemicellulose and lignin create a protective sheath around the cellulose (Carillo et al., 2005). This structure of lignocellulose plays a huge role in inhibiting degradation of the hemicellulose and cellulose structure to monomeric sugars which is necessary to effectively convert biomass into ethanol. So the processing of lignocellulose is therefore essential for the conversion of lignocellulosic biomass to biofuel such as bio-ethanol. Lignocellulose is the renewable biomass produced from photosynthesis. Lignocellulosic biomasses comprise cellulose (25–50%), hemicelluloses (20–30 %), polyphenolic lignin (10–35 %), and other components (Knauf et al. 2004). Cellulose consists of repeating unit of cellobiose. The D- glucopyranose units are linked together by β (1, 4) glycosidic linkage. These are supporting material of cell wall. Cellulose molecule aggregate with each other due to hydrogen bonding and form micro fibrils, which are the building block of cellulose molecule. It consist of two region namely crystalline region and amorphous region. Hemicellulose is a polymer that composed of several different sugars like D- mannose, D-glucose, D- Xylose and D- arabinose and also different uronic acids. Lignins are complex

polymer consists of phenylpropanre units which linked together by ether or carbon-carbon bonds. Classification of lignin is on their structural elements and differs from one species to another species. Besides these lignocellulosic biomass contain a group of chemicals called as extractives which include terpinoids, steroids and phenolic constituents. Some of them are important for plant's defense system.

2.7. *Ipomoea carnea* as a source of lignocellulosic biomass

Ipomoea carnea is also called as pink morning glory, is a species of morning glory (Fig 2). This flowering plant has heart shaped leaves that are a rich green and 6-9 inches long. It can be easily grown from seeds which are toxic and it can be hazardous to cattle, the toxicity is related to the bioacuumulation of selenium species in leaves but mostly in seeds. The stem of *Ipomoea carnea* can be used for making ethanol production. Biomass containing more than 70% cellulose and hemicellulose can be considered as a good candidate for bioethanol production. The lignin content should be less than 20% (Sindhu et al., 2010). *Ipomoea carnea* fulfill all the above criteria for bioethanol production. *Ipomoea carnea* is an important non edible lignocellulosic biomass, which grows widely throughout India. It is non edible due to its high toxin content.

Fig.2: *Ipomoea carnea,* source of lignocelluloic biomass

Ipomoea carnea has also a strong industrial importance as a source of oleanolic acid and carboxymethylcellulose (CMC). *Ipomoea carnea* biomass can be implicated as a substrate for

bioethanol and biogas production (Gamez et al., 2005). Besides high amount of CMC and lignin content recently *Ipomoea carnea* is reported as cellulose catalyzer.

2.8. Cellulase

Cellulase is a complex enzyme which breaks down cellulose to beta- glucose. It is produced mainly by symbiotic bacteria in the ruminating chambers of herbivores. It is produced chiefly by fungi, bacteria and protozoans that catalyze cellulolysis. Several different types of cellulose are known, which differ structurally and mechanistically.

Cellulose is a linear polysaccharides of glucose residues connected by β-1,4 linkages (Fig.3)

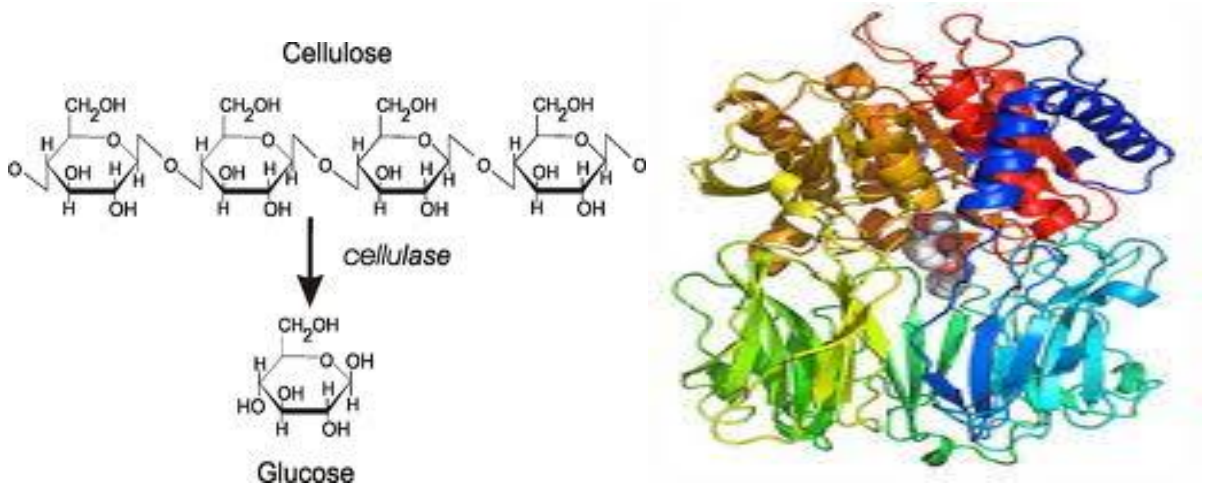

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Fig.3: Cellulase Enzyme Complex (Ref: www. Chem alignment.com)

Cellulase refers to a family of enzymes which act in concert to hydrolyze cellulose. Cellulase are widely distributed throughout the biosphere and are most manifest in fungal and microbial organism. Five different types of cellulase based on the type of reaction catalyzed in endocellulase randomly cleave internal bond at amorphous sites that create new chain ends. Exocellulase cleaves two to four units from the ends of the exposed chain produced by exocellulose, cellobiase or beta glucosidase hydrolyse the exocellulase product into individual monosaccharides. Thus, the endo and exocellulases appear to work in a synergistically or cooperative manner on native cellulose (Karamakar and Ray., 2010).

3. Objectives

1. To isolate cellulase producing bacteria from deer and cow dung

2. To study the optimum time period for enzyme production by bacteria

3. To study the optimum conditions (pH, temperature and metals concentration) for cellulase activity

4. To produce bioethanol from steam and acid pretreated lignocellulosic biomass of *Ipomoea carnea*

4. Materials and Methods

4.1 Collection of Cow and Deer Dung

Deer dung was collected from Jubilee Park (Deer Park), Rourkela and Cow dung from cattle farm near Jagda during the month of January, 2013. The fresh dung was sterile for 20 min and then used for bacterial growth. (Fig 4)

Fig.4: Cow and Deer

4.2. Isolation of Cellulase Producing bacteria from Cow and Deer dung

Serial dilution of cow and deer dung was inoculate in Trypticase Soy Agar (TSA) media (15 g Tryptone, 5 g soytone, 5 g sodium chloride, 15 g Agar/1L d/w) Individual colony was obtained by streaking the culture in same media. Confirmation of cellulose-degrading ability of bacterial isolates was performed by streaking on the Carboxy methyl cellulose (CMC) media with the following composition: 15 g CMC, 3 g $NaNO_3$, 3 g K_2HPO_4, 3 g KCl, 0.5 g $MgSO_4$, 0.5 g yeast extract, 1 g glucose, 17 g agar. Congored(1mg/ml d/w) was used as staining solution and NaCl (0.1M) as a de staining solution. The use of Congo-Red as an indicator for cellulose degradation in an agar medium provides the basis for a rapid and sensitive screening test for cellulolytic bacteria. Colonies showing discoloration of Congo-Red was taken as positive cellulose-degrading bacterial colonies (Wang et al., 2004). And only these were taken for further study.

Table 1: Composition of TSA

Composition	Concentration(g/l)
Tryptone	15
Soytone	5
Sodium Chloride	5
Agar	15

Table 2: Composition of CMC agar

Composition	Concentration(g/l)
CMC	15
NaNO3	3
K2HPO4	3
KCL	3
MgSO4	0.5
YE	0.5
Glucose	1
Agar	17

4.3. Cellulase Enzyme Production

The selected Cellulose Digesting Bacteria isolates were cultured at 37˚C at 150 rpm in an enzyme production media composed of 1 g $NaNO_3$, 1 g KH_2PO_4, 1 g KCl, 0.5 g $MgSO_4$, 0.5 g yeast extract , 1 g glucose and at pH 6.8–7.2. Broth culture after three days of incubation period was subjected to centrifugation at 5000 rpm for 15 min at 4˚C. Supernatant was collected and stored as crude enzyme preparation at 4˚C for further enzyme assays.

Table 3: Composition of enzyme production media

Composition	Concentration (g/l)
$NaNO_3$	1
KCL	1
KH_2PO_4	1
$MgSO_4$	0.5
YE	0.5
Glucose	1

4.4. Cellulase Assay

Total cellulase activity was determined by measuring the amount of reducing sugar. Endoglucanase (β-1-4 endoglucanase-EC 3.2.1.4). Activity was assayed by measuring the amount of reducing sugar from amorphous cellulose. Endoglucanase activity was determined by incubating 0.5 mL of supernatant with 0.5 mL of 2% amorphous cellulose in 0.05 m sodium citrate buffer (pH 4.8) at 55˚C for 30 min. After incubation for an hour at 55˚C, the reaction was terminated by adding 3 mL of 3, 5-dinitrosalicylic acid (DNS) reagent to 1 mL of reaction mixture. In these tests, reducing sugars were estimated spectrophotometrically with 3, 5-dinitrosalicylic acid using glucose as standards (Ghose, 1987). Then enzymatic activities of total endoglucanase were defined in Enzyme Unit. One Unit of enzymatic activity is defined as the amount of enzyme that release 1µ mole reducing sugars (measured as glucose) per min.

4.5. Effect of Time period on cellulase production by bacteria

Different incubation times (24, 30, 36, 42, 48, 54, 60, 66, 72 hours) were employed to study their effect on the cellulase production. The culture filtrates were collected at respective time interval and assayed (Gamez et al., 2005)

4.6. Effect of temperature on cellulase activity

Enzyme Activity was carried out at different temperatures such as (30, 40, 50, 60, 70℃) to study their effect on cellulase activity. The culture filtrates were then collected and assayed (Gamez et al., 2005).

4.7. Effect of pH on cellulase activity

The pH of the production medium was adjusted to 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 8.8 with phosphate buffer to see the highest cellulase activity. The enzyme activity was carried out to study their effect on enzyme production (Gamez et al., 2005).

4.8. Effect of metals on cellulase activity

After getting optimum temperature and pH value different metals such as $MgSO_4$, $ZnSO_4$, $MnSO_4$, $FeCl_2$ and $CoCl_2$ were subjected to study their effect on enzyme activity. They all were taken in a concentration of (5 mM) and assayed (Sommer et al., 2004).

4.9. Collection of *Ipomoea carnea*

Ipomoea carnea was collected locally from Hill Top, National Institute of Technology campus Rourkela, Odisha during the month of December. It was sun dried for one month followed by grinding by using mixer grinder. Powder was stored.

4.10. Pretreatment of *Ipomoea carnea* stems

Ipomoea carnea stems were pretreated with by steam treatment and acid treatment. Steam treatment was given by (autoclaving the slurry (5%, w/v) at 121˚C for 15 mins. Supernatant was discarded and pellet was obtained). Acid treatment (5% H_2SO_4, 140°C for 45 min incubation was given to the pellet obtained after stem treatment). Supernatant was discarded and pellet was obtained (Ghose et al., 2003).

4.11. Saccharification

Enzymatic hydrolysis performed separately from fermentation step is known as separate hydrolysis and fermentation (SHF) (Wingren et al., 2003). In saccharification process, 9.9 ml of solution cointaining 3.3 ml of crude cellulase of each bacterial isolate mixed with 10 g of steam

and acid pretreated biomass incubated in 55 ℃ for 72 hours. After 3 days, saccharified biomass was heated up to 90°C and cooled. Centrifuged at 5000 rpm for 10 mins and supernatant obtained. This supernatant now obtained subjected for fermentation process.

4.12. Fermentation

Saccharomyces cerevisiae grown in glucose yeast extract broth medium for 48hours. 10% inoculums was used for fermentation of medium containing saccharified solution of *Ipomoea carnea* from the previous step and kept for 3 days at room temperature. Ethanol was estimated by gravimetric analysis (Ghose et al., 2003).

4.13. Estimation of Ethanol

Bioethanol produced by separate saccharification and fermentation process was estimated by gravimetric analysis by using following formula.

$$\% \text{ of alcohol} = \frac{1.05 * (SG1 - SG2) * 100}{SG2}$$

Where,

SG1 = Initial specific gravity

SG2 = Final specific gravity

5. Results and discussion

5.1. Isolation and Screening of Cellulase Producing Bacteria

Cellulase Producing bacteria were enriched and isolated by inoculating deer and cow dung on TSA medium and by streaked on CMC agar medium. A total of two bacterial isolates found to be positive on screening media (CMC agar) producing clear zone with Congo red stain (as shown in Figure 5).

Fig.5 Zone of clearance on CMC agar plates produced by cellulase producing bacteria after 48 hours of incubation. The formation of clearing zone around the colonies confirms the secretion of extracellular cellulase.

5.2. Effect of time period on cellulase production by bacteria

Enzyme activity recorded at different time period (after every 6hours interval) revealed that all the two bacteria yielded maximum cellulase production at 72 hours of incubation (Figure 6). The time period was found to influence extracellular enzyme secretion, possibly by changing the physical properties of the cell membrane. Since the cellulase production was continued up to 72hours, it is necessary to study the cellulase production beyond 72 hours.

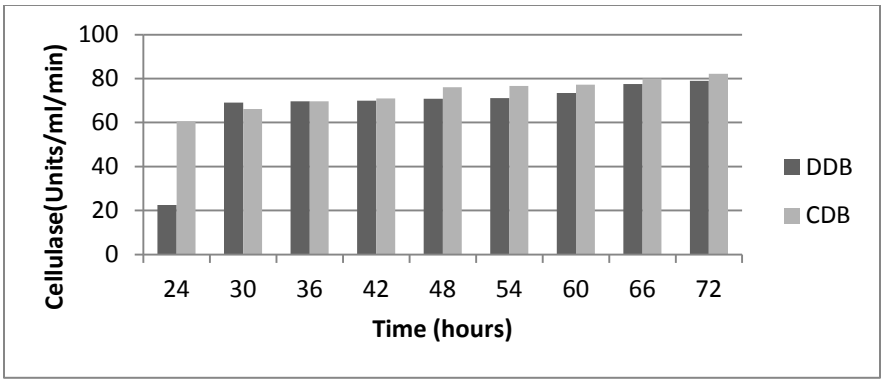

Fig 6: Effect of time period on cellulase production by bacteria

5.3. Effect of temperature on cellulase activity

Enzyme activity recorded at different temperature revealed that the optimum temperature for Deer Dung Bacteria 50˚C and for Cow Dung Bacteria 60˚C (Fig 7), where maximum enzyme production occurs. Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is increased. In every ten degree centigrade rise in temperature will increase the activity of most enzymes by 50 to 100%. Here the enzyme may infer as therostable enzyme as it can withstand temperature up to 50 ℃ to 60 ℃ and shows its maximum activity.

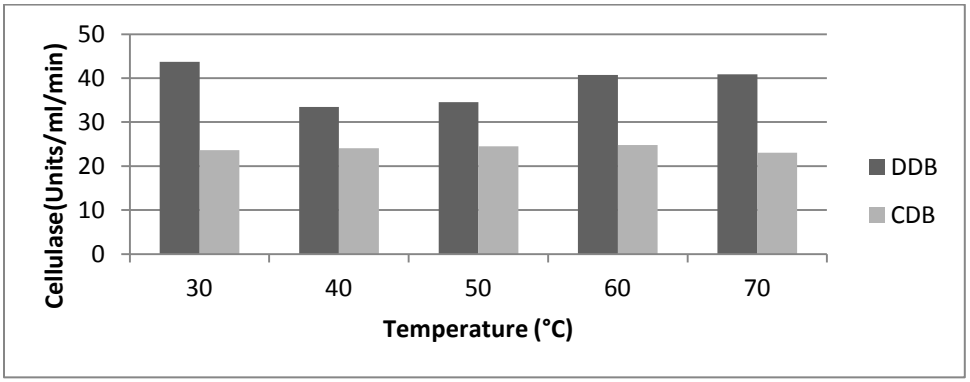

Fig 7: Effect of Temperature on cellulase activity

5.4. Effect of pH on cellulase activity

Enzymes are affected by changes in pH. The most favourable pH value is the point where the enzyme is most active- is known as the optimum pH. The two enzymes were allowed to test in different pH ranging from 3.0 to 8.8. Maximum enzyme activity was observed around pH 3.0 in case of both the enzyme (Fig 8).

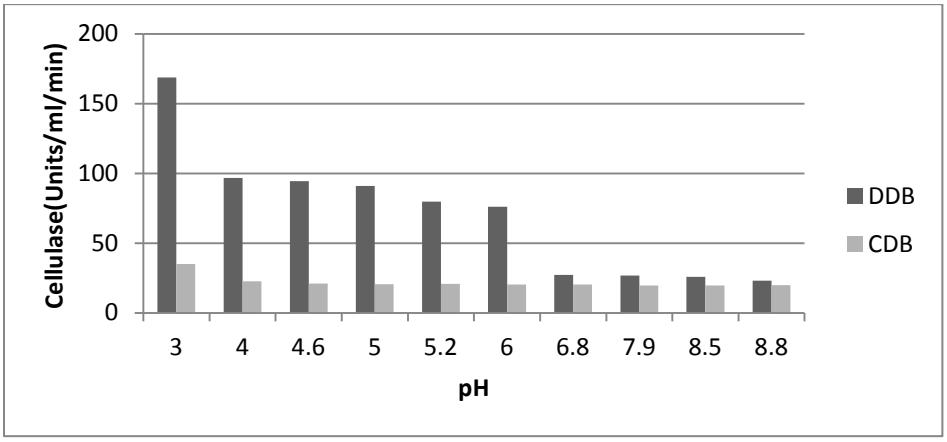

Fig. 8: Effect of pH on cellulase activity

5.5. Effect of metals on cellulase activity

After getting optimum temperature and pH value different metal ions such as $MgSO_4$, $ZnSO_4$, $MnSO_4$, $FeCl_2$ and $CoCl_2$ were subjected to study their effect on enzyme activity. Activity of Cellulase enzyme produced by bacteria isolated from deer and cow dung was found to be increased by the addition of 5 mM $MnSO_4$ (Deer dung Bacteria and Cow Dung Bacteria). It can be inferred that $MnSO_4$ (Deer Dung Bacteria and Cow Dung Bacteria) may act as a cofactor or enhancer of enzyme productivity (Fig .9)

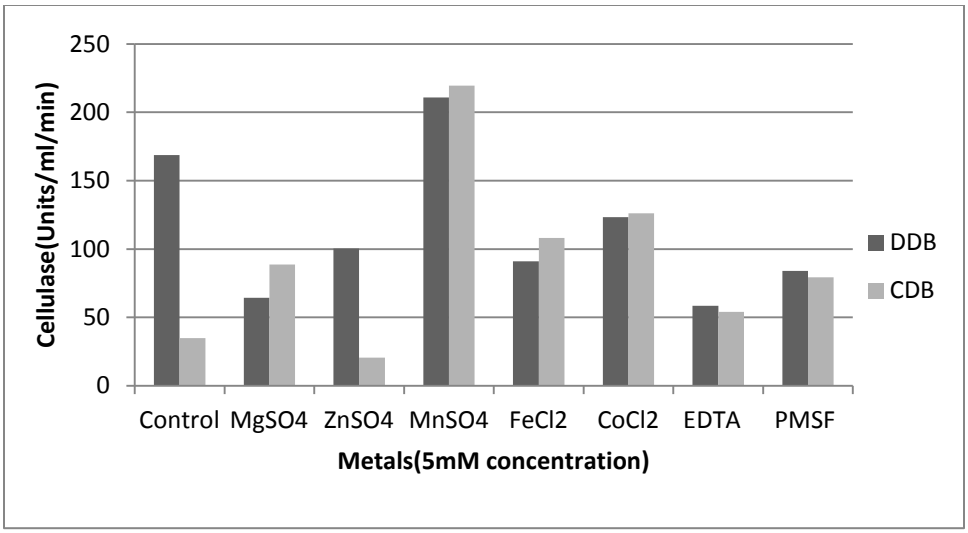

Fig. 9: Effect of metals on cellulase activity

5.6. Bioethanol production

The experiment set up for separate saccharification and fermentation of mixed bacterial culture with *Saccharomyces cerevisiae* resulted in the production of ethanol, estimated by gravimetric analysis and it was 5.52%. The less amount of ethanol production is due to there may be some inhibitor for cellulase enzyme from lignin and hemicelluloses even after saccharification and to maintain yeast growth in its optimal temperature is very difficult at room temperature in summer season.

6. Conclusion

From the present study, it is concluded that bacteria isolated from deer and cow dung (Deer Dung Bacteria and Cow Dung Bacteria) was producing maximum amount of cellulase enzyme. Cellulase enzyme produced by bacteria isolated from deer and cow dung was found to have optimal pH around 3, optimal temperature 50°C for Deer Dung Bacteria and 60°C for Cow Dung Bacteria as optimum conditions. Activity of cellulase enzyme produced by bacteria isolated from deer and cow dung was found to be increased by the addition of 5 mM $MnSO_4$ (for both DDB & CDB). It is possible to produce lignocellulosic bioethanol (5.52%) from *Ipomoea carnea* after steam and acid pretreatment by using Cellulase for Saccharification (72 hours) and *Saccharomyces cerevisiae* for fermentation (72 hours). Bioethanol from lignocellulosic is a globally accepted alternative fuel. The production of ethanol from *Ipomoea carnea* would have the dual advantage of producing energy and serving as an effective method of weed management.

7. References

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