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RESEARCH ARTICLE

COW-DUNG – POSSIBILITY FOR A SUSTAINABLE FUTURE BIOREFINERY IN INDIA PART-I AN OVERVIEW

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ABSTRACT

In India, 69 %-70% population is living in rural areas where cow (*Bos indicus*) is principal cattle. Total Milk producing Cows in India are about 80 million and non-milk producing cows are on the order of 30 million each generates 9–15 kg dung/day. On an average in India from the non-milk producing cows 8×10^7 kg per day cow dung is produced. One of the serious social problems is the waste generated from stray cows and gaushalas which is not only source of solid pollutants but also run off liquid along with gaseous emission, some of which are either hazardous, lethal or mutagenic or even poisonous, or pathogenic in nature. The solutions for this major problem due to either pollution or disposal of solid wastes can be overcome by the synthesis of some of the worth-mentioning products which include bio-plastics, or bio-textiles, α -cellulose, pulp, paper and card boards for sustainable packaging, bio-gas, bio-oils and fermentation products like ethanol, bio-composting and bio-fertilizers (N-P-K), nano-cellulose and MFC, microbial products (bacteria and enzymes), activated carbon, and fillers, bricks, silica, ceramics, and silicon-based semiconductors. In this present paper a review is made on these invaluable products with special emphasis on bio polymers and polymers from the ligno-cellulosic part of the cow dung, which in turn consists of cellulose, hemicellulose, lignin, and sometimes starch also. It is important to mention that based on recent survey the total amount of lignocellulosic part (sum of cellulose, hemicellulose and lignin) in cow dung in India is reported on the order of 52%.

INTRODUCTION

Manure of bovine animal (also named as cow feces, pies, cow pats, cow excreta, cow poo in many countries) is one of the solid wastes which creates environmental pollution (1-9): India is one of the major producers of manure of bovine animal, cow dung in particular which are abundantly available across the country. This paper presents an overview on utilization of this valuable bio-waste, for production of bioenergy - bio-fuel and a host of chemical bye products of industrial importance in one hand and protection of environment through abatement of environmental pollution & global warming on the other (1-9). Cow Dung has the traditional uses as follows:

Traditional uses (10-14): It is well known that people in Indian villages use cow dung for cooking purposes as a source of heat using its cake for direct burning. It is also used in plastering of walls, floors, and partitions in rural homes for providing insulation at some point of winter and also during summer time. Application of smoke generated from the burnt cow dung as insect (mosquito) repellent and ash as cleansing agent for kitchen utensils, making of plant pots and Agar-bati, hard chum laxative, bathing liquid, for medicinal treatment in villages such as inflammatory & antibacterial purposes, for religious functions is an age-old practice (10-14).

Personal Care Products (4-5): Personal care products include eye gel, soaps, shampoos, toothpaste, shaving creams, skin cream, sunscreens, face washes, sticks, bio-fertilizers, mud brick for housing, cheap flower & plant pots, and panchgavya (15-17).

Cow-dung as Biorefinery (18-20): As already indicated, this versatile bio-waste can be employed for production of large spectrum of useful value-added products and bio-fuels or bioenergy (7,23-70); some are substitute of petroleum and petrochemicals or pharmaceutical intermediates or being antibacterial and antiseptic medical applications (21-24). Thus utilization of cow dung can be considered as a perfect sustainable bio-refinery provided these are exploited with environmentally benign and economically viable technology. In brief, the products formed in various platforms include bio-pellets, bio-fuels (22-24,54-57,71), fertilizer, (26-34,36) composting for agriculture, solid-liquid and gaseous fuels (methane and biogas) for heating, energy and electricity production, building material, paper, paper board and cardboard, polymer, adhesives and plastics (58-64), ethylene (used in the plastics industry), ammonia, synthesis gas (25), microbes and enzymes (49-51,72), pentosans, furfural, levulinic acids (41-45,47,53,65), pyrolysis gas, syn gas, activated carbon, fuel pellets, electrode material, semiconductors (21,39—40,46,48,57) and myriad of chemical products including nano cellulose (35,70).

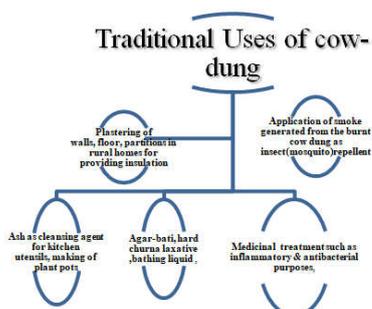


Figure 1. Traditional uses of Cow-dung

This is shown in Figure.2. Its extracted products like cellulose, hemicellulose, lignin and sometimes starch, each one is itself a perfect biorefinery, can produce eco-friendly bio-products of industrial importance (18-23,53,20,36-38).

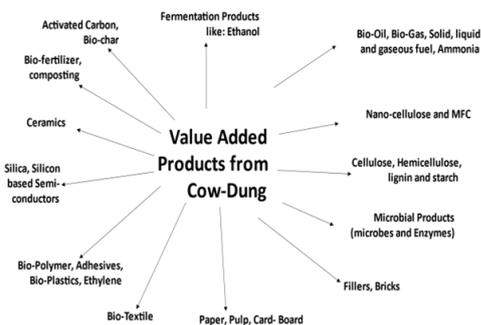


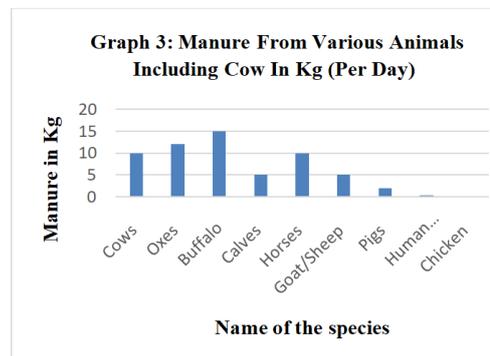
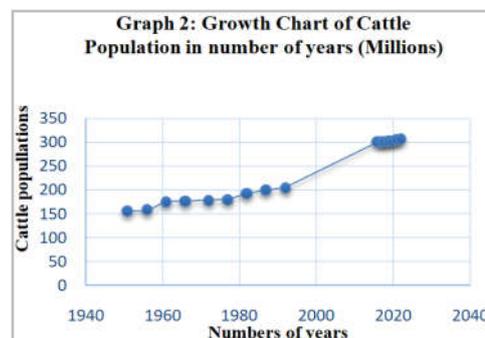
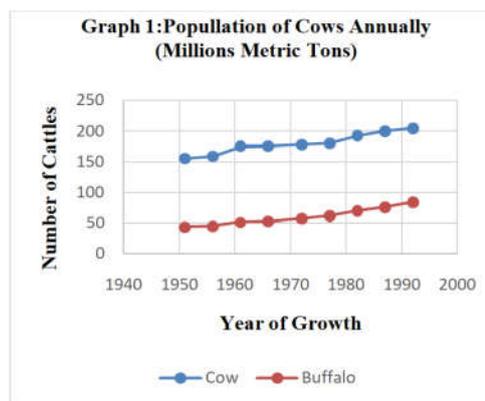
Figure 2. Value added Products

The pollutants out of cow dung include solid and associated emanating odorous gases, liquid run off containing dangerous hazardous contaminants (dioxins, di-benzofuran and chlorophenols & arsenic), pathogens and gaseous pollutants (greenhouse gases NO_x , So_x , methane and higher hydrocarbons). For profitable utilization of this plentiful biomass, conversion technologies, production processes, operations and reactants must be properly engineered and analyzed for sustainable development of the biorefineries taking into account of techno-economics, environmental as well as socio-cultural considerations. In this present paper, among the myriads of bio-products, bioplastics, biopolymer including polymeric adhesives, and bio-textiles are given special emphasis without detailing chemical and bio-chemical reactions involved & methods of manufacture.

Strategies need to be developed looking at the current and futuristic trends with respect to its production and utilization, emphasizing on the societal benefits for pollution abatement, poverty alleviation, income & employment generation for the rural masses, thereby boosting the rural economy. Review on recent development on utilization of this renewable sustainable green resources in India is also attempted. Preliminary analysis on cow dung utilization exhibits a promising avenue for the future bio-refinery. Presently global cattle population is over 996 millions. The ranking in various countries are as under in decreasing order: India >Brazil >China >US > European Union > Argentina > Australia > Russia(1-9). In India 69-70 % population resides in rural areas and has a very large livestock population and ranks first among the livestock including crossbred cattle's holding countries in the world is over 600 million (8). Total population of female cows in India are 190.90 millions. Cow (*BOS indicus*) produces cow dung approximately 9 to 15 kg /day (average 10 kg per day) (7,23,61). On an average 18×10^7 kg per day cow dung is produced in India from the non-milk producing cows. Total live stocks cow dung are 450 million tons. Average gas production is of the order of 40 lit/kg (4,2,11).

Growth of Cows in India (1-3,23): It is reported that there is an increasing trend of growth of livestock population in India. This is evident from the following graphs. Nearly 600 million metric tons of wet dung is produced annually from a livestock population of

about 288 millions (cattle and buffaloes) and considered wet dung is a sustainable resource. Biogas production would be 36 b Cubic M/y. This is evident from in the following graphs (1-3).



Physical Properties and elemental composition of cow dung manure (5-6,8-9): Cow dung is bulky; colour changes from greenish to dark brown to blackish, further darkening on exposure to air and have high ash content. Many investigators (x) determined experimentally physical properties (pH, Electrical conductivity), moisture content, volatile solids, ash content, proximate and chemical analysis, fiber morphology, elemental composition (C,N,P, K, Zn, etc. C/N) and physio-thermal properties (Ignition temperature, Peak ignition temperature) of Indian cow dung had been reported (6). Cow manure is rich in organic materials and in nutrients; contains high level of ammonia as well as potentially dangerous pathogens. 24 number of elements (metals and non-metals) with some traces include nitrogen ,3%, phosphorus,2%, potassium,1% (3-2-1 NPK), Ca, S, Fe, Mg,Cu,Co, and Mn either in combined or free form were studied (-). The indigenous Indian cows additionally include higher quantity of Ca, P, Zn, Si and Cu, than the cross-breed cow(6,8,9) Microelements such as B,Al, Ba,Bi, Cd,Ni,Pb,V etc are also found through ICP-MS. Fasake and Dashora (74) studied on the Characterization and morphology of three types of animal dungs for potential industrial application as Bio-based Fillers. The dungs were obtained from indigenous Cow (IDF), Jersey cow, JDF, & Buffalo, BDF; all of them were fed the same feed, wheat straw. Proximate, and ultimate analysis of raw dung fibres, and their physical characterizations were made. Various analyses were made by FTIR, SEM, Energy Dispersive X-ray Spectroscopy (SEM-EDX)

Table 1. Average Composition of Carbohydrates found in Cow-dung

Cellulose%	Hemicellulose, %	Lignin, %	Total	References
				https://www.researchgate.net>
1.6-4.7	1.4-3.3	2.7-5.7	5.7-13.7	K. Li, Liu, & Sun, 2015 (Parveen Kumar, Diane)
1.6-26.6(Avg.24.2)	1.4-12.8(Avg.12.1)	2.7-13.9(av.11.0)	5.2-53.3(47.3)	K.Li <i>et al.</i> ,2015)2015,Liao <i>et al.</i> 2006, Parveen Kumar <i>et al.</i> 2009; Wen <i>et al.</i> 2004
26.59	11.27	11.24	49.1	M. Barrett, Michael J., Delwiche, & Stroeve(2009)
23.51	12.82	7.95	44.28	Liao, Liu, Liu, Wen & Chen(2006)
21.89	12.47	13.91	48.27	Wen, Liao, & Chen(2004)
26	11	11	48	Zulkifli <i>et al.</i> (2018)
26	14	12	52	Indian cows (5)
29-31.5	21-23.50	11-13	61-68	Fasake & Dashora (2020)
Holo cellulose=35.09		19.02	54.11	Ash=17.4%

Imaging), ICP-MS,(inductively coupled plasma –MS), The average composition of all the animal dung samples was found as:

Cellulose: 29-31.5%, Hemicellulose;21-23,5 %, Lignin:11-13%. However these values were found to be much higher than already reported values from various sources as shown in Table 1.

Cow Dung Ash (39,40): It contains high concentration of alkali compounds, rich in N,K and P with approximate percentages (by weight) 1.1,7.3, and 3.2 respectively and also Cl 2.57%,CaO,30.6 %, K₂O,55.6.(8). Further, it is also reported that N content of some cow dung is 1.7% and sheep dung is 3.75 respectively with C/N =0.25(7).

Chemical composition of Cow dung: Cow manure is produced from undigested grass, straws, tree leaves, shrubs, crop and grain. The total amount of lignocellulosic part (sum of cellulose, hemicellulose and lignin) in cow dung varies significantly as shown in the following table. The average composition of cow dung is shown below; the lignocellulose content is approximately of the order of 52% (41-42) Engineered Technology (27-37, 72). The inputs including feed stock and technology are engineered in such a way so that it can integrate with the best available process technology for the development of biorefinery- environmentally benign for the environmental protection, sustainable, renewable & economically viable. The process engineering includes preparation of manure, followed by depending upon wet or dry processing- the pre-treatment technology, downstream processing, refining with further treatments and finally purification of value-added bio-products (http Ref// bio-process.co. Biorefining –systems) (Figure 3).

Renewable Fuels-Ethanol, Butanol, Hydrogen and Biodiesel etc.

Chemicals- Xylose, Glucose, Arabinose, Total phenol, Acetone, Furfural, 5-HMF, 2-MTHF (2methyltetrahydrofuran), Ethanol, Butanol, Propanol (Propane diol), Ketones, Vanillin, Syringaldehyde etc.

Organic Acids - Volatile organic acids like Acetic, Butyric, Formic, Propionic and other short-chain fatty acids; non-volatile acids like Lactic, Succinic, Gluconic, p-Hydroxy benzoic acids, Vanillic acid, Syringic acid, p-Coumarilic acid, Ferullic acid etc.

Bio-Energy
Lignite, Charcoal, Methane, Bio-gas, Bio-char, Bio-oil, Pyrolygneous acids, Heat Electricity
Food and Feed- Single cell protein, Fat/fiber/ mono- and poly-saccharides such as glucose, fructose, sugar etc

Figure 3. Engineering Technology Process by-products are classified in to five categories

Chemicals from biomass using thermos-chemical platform are given elsewhere (7,22-23,46,72)

3 Cow-dung as a Biomass conversion technologies and processes: The details of the processes and products formed from different technologies are given in Figure 4(7).

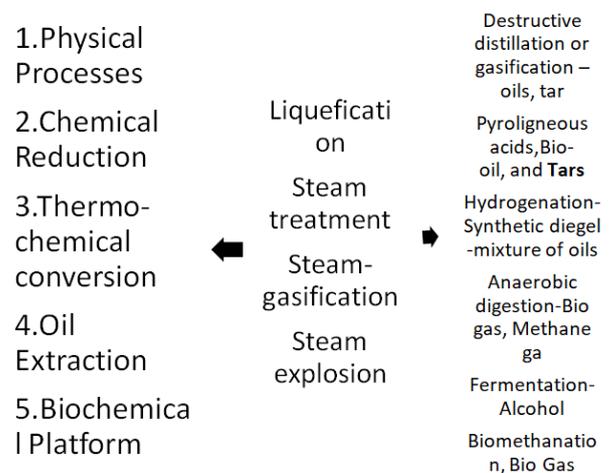


Figure 4. Biomass Conversion Strategies and Products (52-54)

Pre-treatment technology for conversion of Cow-dung: This includes a) physical-mechanical, b) physio-chemical (Ammonia fibre explosion (AFEX), carbonic acid, or SO₂), (25) c) chemical (pre-hydrolysis, hydrolytic medium - water, acids, alkaline extraction, wet oxidation and organo-solve). Most common methods are steam explosion and dilute acid pre-hydrolysis though carbonic acid and alkaline extraction should give best performance (47). Steam explosion process employs high pressure 0.69-4.83 MPa at high temperature 160°C-260°C (most frequently 180-200°C) for several seconds, followed by sudden decompression to atmospheric pressure causing material explosion. Maximizing the pre-treatment process using steam explosion technology is accomplished by optimizing process parameters such as temperature, pressure, residence time in to the steam reactor, use of acid catalyst, susceptibility of the pre-treated biomass to bioconversion and process design (28). Addition of H₂SO₄ or carbonic acid is often adopted to reduce the concentration of inhibitors as well as to enhance hemicellulose solubility. Both dilute and concentrated acids are used in single or multiple stages. A solution of H₂SO₄ (0.5-1.0%) was treated at about 160-190 °C for approximately 10 minutes which gives easy hydrolysis of hemicellulose and release monosaccharides like xylose, arabinose, mannose and galactose along with many other compounds from lignocellulosic matrix. This is then cooled, part of acetic acid formed, and much of sulphuric acid are removed followed by neutralization and the setting to pH 8 to 10 before enzymatic hydrolysis and fermentation. Two stages hydrolysis is carried out to get maximum sugar yields, the first stage is operated in milder conditions to hydrolyse hemicellulose, while the second stage is optimized to hydrolyse the more resistant cellulose fraction. Liquid hydrolysates are recovered from each stage, neutralized and fermented to ethanol (46,47,54-57). In concentrated acid hydrolysis

process recrystallization of cellulose occurs followed by dilute acid hydrolysis to produce sugars. Experimental results with dilute acid at a temperature of 180-200°C for wood biomass, alkali treatment and steam explosion for bagasse (43, 47-46) have also been reported. The experiments conducted on bagasse hemicellulose hydrolysate based on statistical 2⁴ full factorial design with four replicas at the centre point. The effects of pH, temperature and hydrolysate concentration on removal of volatile and non-volatile compounds from hemicellulose hydrolysate treated with activated carbon were examined.

Biochemical processes-a Biological Platform

Enzymatic Hydrolysis (46-47,49-52). Hydrolytic hydrolysis is followed by Enzymatic Hydrolysis. This is achieved using cellulases, hemicellulases which are usually a mixture of group of enzymes such as endoglucanases, exoglucanases and β -glucosidases etc. This removes cellobiose and hydrolysing it to monosaccharides e.g., glucose at a temperature between 50-60°C. The process is also called saccharification. For degradation of hemicellulose, the enzyme is consisting of exoxylanase, endoxylanase, and β -xylosidase (which splits xylose and others short chain xylobioses)

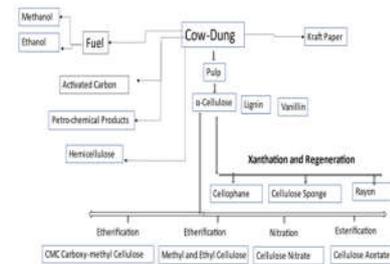
Fermentation: Fermentation occurs in presence of yeast (*Saccharomyces Cerevisiae*) at a temperature of 30°C. Biological treatment includes fermentation & bioprocessing of downstream products, product separation & recovery and their purifications. Simultaneous saccharification and fermentation (SSF) can also be done in the same reactor. Conversion of glucose and xylose using *Zymomonas mobilis* known as Simultaneous saccharification and co-fermentation (SSCF) is also possible separately or in the same reactor (28-29,31-34). Pre-hydrolysis of cow dung can be done either in single stage or in two stages. The two-stage acid hydrolysis of bagasse was carried out by HCl (diluted and concentrated) under atmospheric conditions and obtained production of xylose and glucose streams which subsequently be fermented to ethanol (33,43, 46-47). Purification for dehydration to products either through distillation or mechanically separation process.

Bio-Products from Cow-dung: Bio –Fertilizer, composting, vermicompost and organic manure (26-27,31-33). Cow dung has been an important bio-fertilizer as it contains N, P, K. It may be fortified and converted to organic fertilizer which improves soil properties, nutrient uptake and yield to make it more profitable. Composting can also be processed by mixing with other biomass or solid wastes, ash or biogas slurry. The composting machine and procedure are well developed. This is a case of Aerobic digestion which involves biological decomposition of organic wastes /residues i.e., the conversion of organic substrates by microorganisms in to utilizable forms in presence of air under controlled conditions to result in release of C,N,P,K etc. & to produce gases, single cell protein, fertilizers etc.

Spectrum of useful Plastic products (58-64)

Biological Platform: Microbial Based technology from Cow manure to plastics. Plastics obtained from bio-resources are called bio-based plastics. It is of two kinds: bio-plastics (synthetic, semisynthetic and non-biodegradable), and the other biodegradable plastics (fully biodegradable, partially bio-degradable) may be naturally obtained, biologically synthesized through enzymes or chemically synthesized. Biodegradable Polymers include polylactic acid, poly(lactic-co-glycolic) acid, Poly (caprolactone), Polyglycolic acid. Example of synthetic Bio-degradable polymer is Polydioxanone (PDO,PDS) or Poly-p-dioxanone. Bioplastics can be produced by microorganisms also from Cow Dung (Erik Coats, University of Idaho, USA) (75). It is indicated that 10-12 gallon of wet manure can be transformed into up to 5 lbs of plastic each day. Examples such as Polyhydroxyalkanoate (PHAs), such as Polyhydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV) are the most common biodegradable plastics (75). Process of Manufacture developed by Erik Coats:(75). According to Erik Coats the development of biopolymer starts with

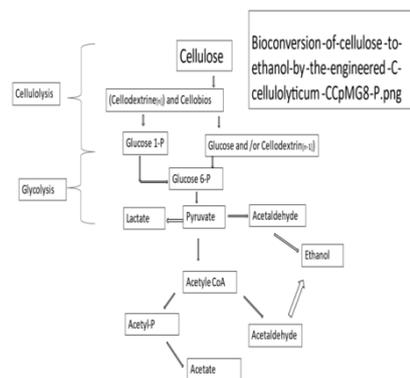
fermentation of the carbohydrate –rich manure to produce organic acids which become food on naturally occurring soil bacteria which is harvested from local waste water treatment facility. The bacteria stores excess organic acids as PHA. When the bacteria are killed and dried, the PHA can be separated out as a crumbly, white raw plastic. It leaves a relatively small carbon footprint, uses natural materials and relies on a resource which is environmentally hazardous and farmers want to get rid of. Alternatively, after drying the manure, separating the cellulose which can be converted to paper products while the wetter parts contain acids that are used to create a natural liquid plastic. The liquid part of the fermented manure is used for plastic production whereas the solid part can be processed in anaerobic digester for energy production vis a vis electricity generation.



Cow-Dung Chemicals

Figure 5. Production of different types of Chemicals from Cow-dung

With proper processing, PHA can be used in myriad of products, such as it can be applied to single –use materials like packaging as well as products for which biodegradability comes in handy, like planter pots or erosion control mats. There are ample possibility of producing another polymer, polylactic acids (PLA), and other biodegradable plastics. These can be made from fermented plant starches. Other bioplastics (biodegradable or bio-based plastics) can also be produced from biomass or renewable plant resources. Some may be non-biodegradable plastics or plastics with some degree of biodegradability. Singhvi and Gokhale (38) produced Poly lactic acid from cellulose Substrates (cellobiose, cellobiose, α -cellulose, starch) through simultaneous saccharification and fermentation (SSAF) using *Lactobacillus delbrueckii* or *L.lactis* RM2-24 and *L.rhamnosus* strain CASL Microbial fermentation(+)- or D(-)lactic acid produced by bacterial fermentation of carbohydrates using homolactic organisms. However, DL-lactic acid is produced by chemical synthesis as shown in Figure 6. The scientist also developed bioconversion of cellulose to lactate using cellulolysis and Glycolysis through Entner Doudoroff pathway. (48). This metabolic engineering is based on enzyme assisted chemical reactions, catabolizes into pyruvate and then to lactate (Figure 7). The *C. cellulolyticum* converts cellulose to ethanol with excess pyruvate through bioconversion route. To direct the excess pyruvate Carbon cycle of polylactic acid can be written in Figure 6. (54-57)



P=phosphate, ox=oxidation, red=reduction, HS-CoA, coenzyme.

Figure 6 Bioconversion of cellulose to ethanol by engineered *C. cellulolyticum*CCpMG8.;(54-57)

Table 2. Types of thermoplastic resins, their trade names & other products derived from cellulose (59-64).

Thermoplastic Resin and the process of organic synthesis	Trade Name (62)
Cellulose Acetate (CA)	Kodapak, Tenite, Plastac ele
Cellulose Propionates:	Fortice I, Reed
Cellulose Acetate (fully recyclable), biodegradable	Butyrates Tenite II, Kodapak II
Ethyl cellulose	Ethocel, Soplascoc, Campco
Enzymes	Cellulase. (50,66-70)
Nitration	Cellulose nitrate (Degraded by Fungi)
Acetylation	Cellulose Acetate, propionate, butyrate. CAP, CAB
Etherification	Methyl-, Ethyl-, benzyl-cellulose, Carboxy methyl cellulose (CMC) (carboxymethyl ether of cellulose) ethyl cellulose (ethoxy ether of cellulose) (biodegradable and biocompatible)
Xanthate process	Cellulose Xanthate
DO	Rayon (regenerated cellulose)
Cyanoethylation	Cyanoethylated cellulose
Thiazolidine	Thiazolidine Cellulose

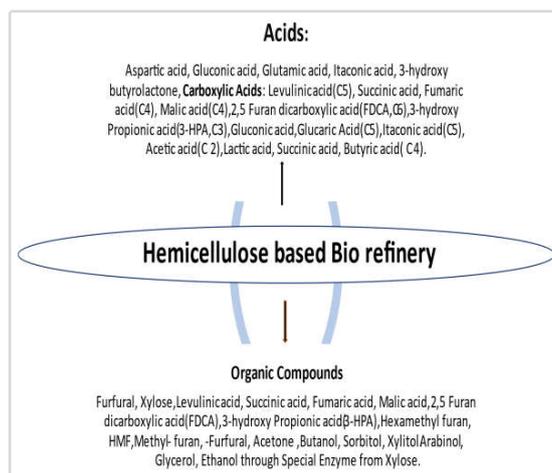


Figure 11. Catalytic fast pyrolysis of furan conversion to gasoline range aromatics and olefins; benzofuran and allene as intermediates (Groups of George Huber, Scott Auerbach, and Wei Fan, Univ. of Massachusetts) (71)

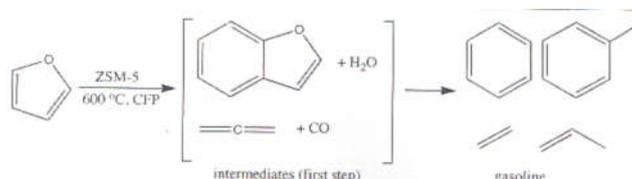


Figure 12. Xylose metabolism of recombinant Zymomonas mobilis (57).

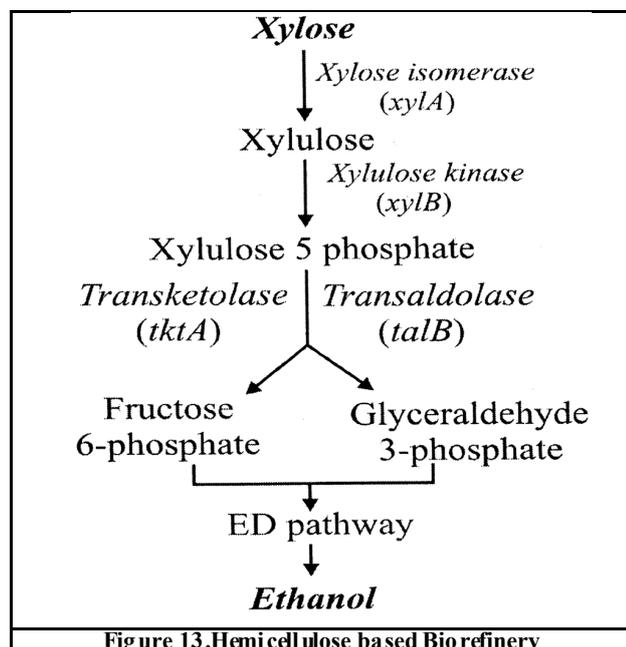


Figure 13. Hemicellulose based Bio refinery

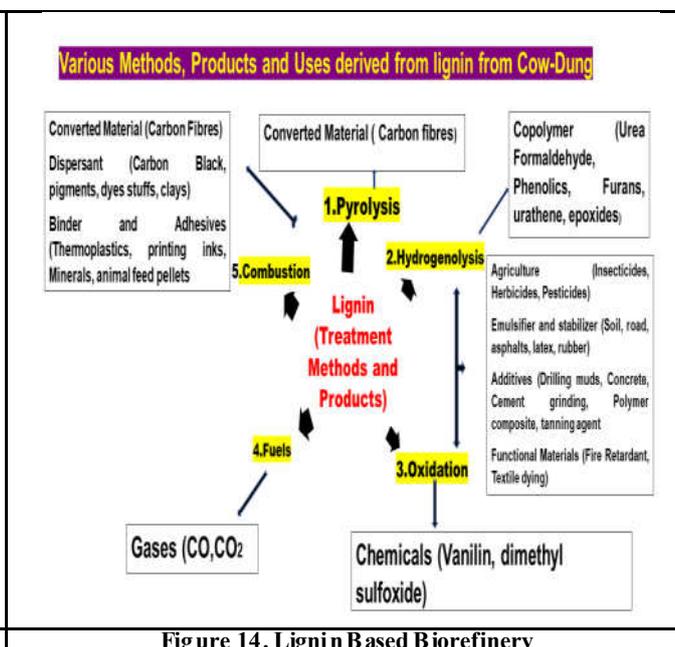


Figure 14. Lignin Based Biorefinery

Lignin based products based on Sulfonation are

- Sulphonated alkali lignin
- Sulfito lignosulphonates to increase tertiary oil recovery in “pumping out” oil wells to replace synthetic detergents.

Both 1 and 2 are marketed as dust stabilizers, asphalt emulsion stabilizers, dispersing agents, binders for various substances, drilling fluid additives, etc., vanillin from sulfite waste liquor, fluid-bed hydrocracking and dealkylation to produce phenols, and benzene. Dimethyl sulphide, Dimethyl sulfoxide (DMSO), Methyl mercaptan are the other products which can be extracted from the waste liquor. Lignin plastics binder is obtained by treatment of high pressure steam up to 8.3 MPa. Various methods, applications and products derived from lignin extracted from cow-dung are given in Figure 14(7,22-23,72).

CONCLUSION

Biorefinery based on Cow dung can be a profitable & sustainable industry in India and some other Asian Countries as astonishingly myriads of chemicals can be obtained from this green resource. Bio-based plastics and Bio-degradable Plastic like molecules and Nanocellulose have a wide range of applications, from cleaning of oil spills to usage in children's toys. Some new cellulose based material can also replace some petrochemical-based products and is very likely to be cheaper than most other kinds of high-performance nanoscale materials. It can not only provide great solution for air pollution with emission of obnoxious gases, safe disposal of hazardous wastes and soil pollution, relieving partly from global warming and carbon footprint, but also replace single use plastic, and provides employment to the rural people, boosting rural economy. However technological assessment of engineered technology using very practical route of converting waste resources to eco-friendly value-added products is an imperative necessity for implementation in practice.

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