#### **Abstract**

Bio-gas coupled with conversion of its waste bio-slurry into bio-fertilizer would benefit India in the field of both energy and agriculture. The Government seems to have made up the mind to move in this direction. The newly floated schemes are a clear indication. When 1 kg of biogas is produced, nearly 100 Ltr of waste bio-slurry is also produced. This bio-slurry is actually "Brown Gold", which can give up to 5X returns to bio-gas owners as compared to the bio-gas. Experts calculate the potential of bio-slurry to save on imported chemical fertilizers to be to a tune of INR 6000 -11,000 Cr every year. However, our work on value addition of biogas slurry suggests that it could be at least 10 times more. A simple model of operations which is "tested at scale", points out, that at least half of this sum earned by the bio-slurry can go into the pocket of farmers - those farmers who setup and effectively run a household biogas plant of 2 m<sup>3</sup> capacity. This economics of bio-slurry will build a strong circular economy. It will also pave new ways to raise the initial capital for bio-gas plants and reduce the subsidy burden on Government.

**Keywords:** Bio-gas, bio-fertilizer, bio-slurry. Waste management.

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### I. BIOGAS BASICS

Microbial process called anaerobic digestion breaks down organic waste materials such as animal manure, crop residues, and food waste to produce a mixture of mainly methane and carbon-di-oxide, which is called as biogas. As per the data reported in 2018, the total biogas production in India is 2.07 billion m<sup>3</sup>/year, which is only about 5% of the actual potential which is estimated to be in the range of 29–48 billion m<sup>3</sup>/year (1).

Biogas generally consists of methane (50–75%), carbon dioxide (25–50%), and smaller amounts of nitrogen (2–8%). Trace levels of hydrogen sulphide, ammonia, hydrogen, and various volatile organic compounds are also present in biogas depending on the feedstock. This can be used as it is for cooking, heating, steam generation, electricity generation purpose. When methane is purified from this gas mixture, it can then be compressed and filled in bottles. This is called as CBG (compressed bio gas) or LBG (liquid bio gas). CBG can be transported, mixed with LNG, used as fuel for vehicles, or used as a pipeline gas.

### II. BIOGAS- IMPORTANCE IN THE INDIAN CONTEXT

Cattle dung has been recognized as the chief raw material for bio-gas plants. Many other materials like night-soil, poultry litter and agricultural wastes, non edible plants, and industrial wastes e.g. press mud from sugar factory, canteen waste etc can also be used to make biogas. In India such biomass is abundant, and at times faces issues of disposal without affecting the environment. At the same time almost 50% of our demand for Liquefied Natural Gas (LNG) has to be met from imports. Biogas can replace LNG. Therefore utilising the waste to create wealth in the form of biogas is important.

Biogas is a renewable energy source. Moreover, it is directly produced at the rural end where it is actually consumed. This is decentralized production. It is particularly attractive as it cuts the costs of distribution. Energy produced at the last mile of utilization adds advantages of circular economy. All these aspects have been put forth with facts and figures by the Minister for Fisheries, Animal Husbandry and Dairying, Hon. Shri. Giriraj Singh in his article "Annadata Se Urjadata".

Biogas is being promoted in India since 1971 through various Governmental programs. Hon Prime Minister of India, Shri Narendra Modi established a separate ministry in the name of "Ministry of New and Renewable Energy" (MNRE) on November 30, 2015. MNRE is key in promoting biogas, apart from other renewable energy projects. There seems to be a change in the approach, sense of urgency, and cooperation between several other concerned ministries to take the "biogas" projects to the next level. The new scheme will enable the biogas projects as well as the country to reap the real potential benefits of organic waste management, biogas, and biofertilizers.

# III.CURRENT EFFORTS TO PROMOTE BIOGAS IN INDIA AT GOVERNMENT LEVEL

1. Ministry of New and Renewable Energy: This ministry promotes establishment of biogas units under its Societal development initiative named as "New National Biogas

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and Organic Manure Programme (NNBOMP). The objective of this scheme is to provide clean cooking fuel for kitchens, lighting, and meeting other thermal and small power needs of farmers/dairy farmers /users including individual households. The new scheme couples biogas with organic manure system based on bio-slurry from biogas plants. The scheme supports biogas plants in rural and semi-urban areas by setting up of small size i.e. from 1 to 25 Cubic Meter capacity.

2. Ministry of Fisheries, Animal Husbandry and Dairying: National Dairy Development Board (NDDB) under this ministry has been working on biogas for over 5 years. Their key specific additional objective was to provide additional income to the dairy farmers and improve the sustainability of dairy farmers. NDDB has formed clusters of biogas plants under the dairy cooperatives – some 30 clusters were proposed (2019). These are at various stages of implementation. They are running a manure management program where in Sudhan brand of biofertilizers are made from the 'biogas waste slurry'. It is expected that the farmers will save/earn in excess of Rs 3000 per month by implementing biogas and sale of biogas waste slurry through the 'manure women co-operative' (3). The article calculates that bio-slurry can replace INR 6000 Cr worth chemical N, P, K, Zn, Fe, Mn, and Cu. Swasti Agro and Bioproducts Pvt Ltd did work with NDDB on this manure management program in two ways. We provided technical material for formulating the Sudhan products of different strengths that were being manufactured in 2019-20. We also worked on 'performance evaluation of the Sudhan products' and 'market development' for the same.

NDDB on July 25, 2022 launched a subsidiary "NDDB Mrida Ltd" with the objective of providing farmer centric cattle manure management solutions, establishing end to end manure value chain (MVC) which includes installation of household to industrial scale biogas plants based on dung feedstock and further providing digestate management solutions and consultancy to different stakeholders as per need.

- 3. Ministry of Jal Shakti: This ministry is supporting GOBARdhan Yojana under "Swachh Bharat Mission Grameen-Phase II". According to Swachh Bharat Mission communication document, GOBARdhan aims to support villages in effectively managing their cattle and biodegradable waste. Department of Drinking Water and Sanitation is working with Ministry of New and Renewable Energy, Ministry of Petroleum and Natural Gas, Department of Animal Husbandry and Dairying, Department of Agriculture, Cooperation and Farmers Welfare, Department of Agricultural Research and Education, Department of Rural Development, state governments, public and private sector institutions and village communities to give this a shape of "Jan Andolan" so that community collective action on GOBARdhan is achieved. It is expected to promote community awareness and ownership, and help villages manage cattle, agro residues and other biodegradable waste. Department of Drinking Water and Sanitation supports every district with technical assistance and financial support of up to Rs. 50 lakh per district to achieve safe management of cattle and biodegradable waste, help villages convert their waste into wealth, improve environmental sanitation and curb vector-borne diseases.
- **4. Ministry of Chemicals and Fertilizers:** The ministry issued a circular on June 28, 2023 the decision of CCEA to supports biofertilizers developed from 'biogas waste slurry. The Cabinet Committee on Economic Affairs (CCEA) chaired by Prime Minister, Shri Narendra Modi today approved a unique package of innovative schemes for farmers. The

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committee approved package consisting of innovative incentive mechanism for the restoration, nourishment, and betterment of the mother earth. Market Development Assistance (MDA) scheme in the form of Rs 1500 per MT to support marketing of organic fertilizers, viz., Fermented Organic Manures (FOM)/Liquid FOM/Phosphate Rich Organic Manures (PROM) produced as by-product from Bio- gas Plants/Compressed Biogas (CBG) Plants set up under umbrella GOBARdhan initiative.

5. Ministry of Petroleum and Natural Gas: The ministry aims to establish an eco-system for production of Compressed Biogas (CBG) from various waste/biomass sources in the country through a scheme called SATAT meaning 'Sustainable Alternative Towards Affordable Transport'. The scheme promotes converting the biogas into compressed biogas, thus bringing the decentralized energy into centralized main frame. As per the official data as of March 31, 2023; There were 46 CBG plants operational, and they provided 16164 Mt of CBG to the centralized main frame. There are 4090 companies that have signed Letter of Intent to produce CBG in the near future. India imported about 20 million Mt of LNG in the year 2022-23, and now Government has made it mandatory to add 5% CBG to the LNG. This will further boost the biogas industry.

It is expected that organic fertilizers based on biogas waste slurry would help addressing the challenge of management of crop residue and problems of 'parali' burning. This will also help in keeping the environment clean and safe and at the same time provide an additional source of income for farmers. Farmers will get organic fertilizers (FOM/LFOM/ PROM) at affordable prices. This initiative will facilitate implementation of Budget announcement of establishing 500 new waste to wealth plants under GOBARdhan scheme for promoting circular economy, by increasing the viability of these BG/CBG plants.

The Economic Times, quoting Indian Biogas Association, has projected the significance of this MDA scheme. It states the Market Development Assistance (MDA) Scheme will increase organic fertilizers production from Gobardhan Plants and will cut 96 lakh tonnes chemical fertiliser import and reap benefits worth Rs 11,000 crore, according to Indian Biogas Association (IBA).

Bio-slurry from biogas plants, and the bio-fertilizers developed from this bio-slurry are getting clear prominence in the bio-gas projects.

## IV. CHALLENGES IN PROMOTING AND IMPLEMENTING BIOGAS PROJECTS

India has a vast experience of promoting and running biogas plant for over 5 decades. So far we have only reached about 4% of its potential, which means there are difficulties. The experience from these exercises has been recorded, analyzed and the barriers in establishment and / or smooth running of biogas plants have been delineated.

Shivika Mittal *et al* have recently (2018) reviewed the barriers in disseminating the biogas technology and installation of plants within rural as well as urban India (1). Not only that this is a review of earlier work along with exhaustive citations, but also includes data generated from interviews with 8 categories of stakeholders of the biogas value chain. As a summary of this work, at the top level, barriers have been divided into following broad

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categories: (i) Financial/economical, (ii) Market (iii) Social and cultural, (iv) Regulatory, (v) Technical & infrastructural, (vi) Information.

While setting up of a new biogas plant, cost of the installation is definitely a concern. The Government schemes are to support beneficiaries that fall under specified criteria. The classes of the society that can't meet the prescribed criteria are often deprived of biogas units for its installation cost. The prospective beneficiary is often convinced about the advantages of biogas as a fuel. But the same dung that is expected to be used for biogas, is conventionally used as fuel on drying, or used as manure after composting. These two conventional uses of dung often compete with the resource being offered for a new application.

Under-feeding of inputs or feeding in wrong ratios either results in suboptimal performance of biogas plant or formation of scum, making installed plant completely dysfunctional (1). We have come across biogas plants of two different makes installed in the same village which consume same amount of dung but the ratio of water to be used is different. Drop in temperature reduces the production of biogas. The beneficiaries often lack information about such technicalities and operations.

Although, the beneficiary farmers are told that the waste bio-slurry coming out of the biogas plant is a great fertilizer; the farmers don't have tools to handle it. Biogas waste slurry contains 10% solids (dry basis) and can't be pumped using the devices available with the farmers. We have seen that the waste slurry coming out of the biogas unit is often dumped in a pit. When the pit is full, because the users don't have efficient means to dispose the slurry, they reduce the addition of input of dung biomass. This is one of the main reasons of converting a successfully installed and working plant into a completely dysfunctional project.

Debadayita Raha *et al* surveyed implementation of decentralised biogas plants establish in Assam between 1998 and 2012 (2014). They report that 20% of the plants were non functional. Out of the working once, about 40% did not utilize the bio-slurry and were simply throwing away the bio-slurry coming out of the biogas plant (2).

The recent initiatives of various ministries of Government of India that we discussed above, have all focussed on both financial support to establish the biogas plant, and use of bio-slurry in agriculture. This use of bio-slurry as a bio-fertilizer will minimize the competition of the dung raw material being used for composting. Also, any extra income from the bio-slurry will make the biogas projects economically robust.

While working with NDDB on their manure management program (3), we at Swasti Agro and Bioproducts Pvt Ltd have designed six functionally distinct liquid products and one solid product that can serve various functions of biofertilizers for the farmers. We also evolved different operational models for utilization of bio-slurry, that can give farmers an additional income of Rs 2000 - 15000 per month from the preliminary processing of bio-slurry (about 3000 Ltr) coming out from a domestic bio-gas plant of  $2 \text{ m}^3$ .

### V. BIO-SLURRY: A HIGHLY VARIABLE MATERIAL

Fermented waste liquid coming from biogas plant is also known as biogas slurry or bio-slurry. This is a by-product generated during the production of biogas. The bio-slurry contains (i) water, (ii) undigested solids, (iii) microbes, (iv) bio-chemicals produced by microbes, (v) mineral elements. No two bio-slurry samples can be same with respect to qualitative and quantitative aspects of all these five parameters. The composition of the bio-slurry depends on very many parameters such as quality and contents of biomass fed, temperature, design of biogas plant, operation parameters such as biomass: water ratio, retention time in days, consistency of operations, handling of the bio-slurry coming out of the bio-gas plant, etc. The variations in the contents of the four parameters of bio-slurry (except water) are discussed below.

The undigested material present in the bio-slurry is typically plant fibres. The efficiently running biogas plant that uses animal dung as starting biomass typically contains between 5 and 10% of solids on dry basis. Solids could be more in the bio-slurry obtained from a bio-gas plant not operating effectively. When the bio-slurry is stored in pits for a long time, further degradation of biomass continues. Also some solids settle down. Under such conditions the slurry obtained has as low as 1% or even less of solids on dry basis.

The pH of the bio-slurry is highly dependent on the biomass used for biogas production (4). When food waste or fruit waste is used, the typical pH of the bio-slurry ranges between 3.0 and 4.5. When animal dung is used the typical pH of the bio-slurry is between 6.0 and 7.8. Often this could reach up to 8.5 due to high ammonia formation. While the new biogas unit stabilizes its gas productivity, pH of the bio-slurry may drop to 5.0. However, at this stage the bio-slurry is not being taken out of the biogas unit. For the same biomass and plant operating conditions, pH of the bio-slurry is a function of retention time. The pH drops when the biomass fed to the plant is retained for more time in the gas plant before being discharged as bio-slurry. Needless to say that the pH of the bio-slurry stored in the pits is also lower than the freshly derived bio-slurry.

Li Sun *et al* reported (2016) the microbial community structure in industrial biogas plants. They reported the analysis of the bacterial communities in the ten industrial-scale biogas plants. The study was done using 454 pyro-sequencing. They found a total of 36,523 types of microbial cultures (as identified by independent sequences after quality trim and chimera check), with a range from 2573 to 4915 sequences (types of unique microbial species) per sample (5). This is one exhaustive study which shows, that no two bio-slurry samples will have same microbial content. From the perspective of further processing of this bio-slurry to organic fertilizers, it is essential that it does not contain any pathogenic microbes. Different studies have shown that the bio-digestion in the bio-gas plant which reaches a temperature of 45° C, often loses most of the pathogenic bacteria, if the retention time is 60 days. Meaning that harvesting of immature bio-slurry for the production of bio-fertilizer could be potentially dangerous.

There is no significant data about the biochemical composition of the biogas waste slurry or bio-slurry in terms of contents of organic acids, microbial metabolites, enzymes, sedarophores, microbiostatic or anti-microbial agents etc. These could be very useful in determination of application potential of bio-slurry as agricultural input. At macro level, on element basis bio-slurry is generally supposed to have (i) Nitrogen (N) 0.5 - 2.5%, (ii)

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Phosphorus (P) 0.2-1.0%, (iii) Potassium (K) 0.5-2.0%, (iv) Carbon (C) 2- 10% and (v) organic matter 5-15%. Along with these it also contains secondary and micro-nutrients in traces (5). The nitrogen present in the bio-slurry is highly variable, mainly because it is highly unstable and can leach rapidly as ammonia gas while the bio-slurry is being handled, stored, and processed. Thus nitrogen level as high as 2.5% of the fresh bio-slurry can rapidly come down to less than 0.5%.

It is a great challenge in itself to use such a variable and undefined starting material to produce agri-inputs that are expected to perform and give consistent results. Unless the bioslurry based products perform consistently, they will not be adopted by the farmers, and the projected returns from the bio-slurry will remain a myth. We will consider strategies to standardize to productize the bio-slurry to get consistent desired results in the coming sections.

# VI. SOME CASE STUDIES ABOUT EFFECTS OF BIO-SLURRY IN AGRICULTURE

There is a huge data available on how bio-slurry can be useful in improving the crop performance and yield. Some such prominent data is reviewed below.

Book entitled "Bioslurry: A supreme Fertilizer" describes positive effect of slurry on an exhaustive list of 51 crops which include staple foods, fruits, vegetables, pulses, and other crops. Most of the study describes results of two plot demonstrations when solid Bio-slurry compost (most probably equivalent to FOM defined by FCO in India). The typical quantity of bio-slurry compost used in these studies is around 15-20 Mt/ha but ranges from 10-40 Mt/ha. Typical yield increase over the control plots reported is between 20-35%, but ranges from 7-54%. Some qualitative claims of building disease or insect resistance have also been made (6).

A review of scientific literature on the co-product of biogas production is published by Food and Agriculture Organization of the United Nations [FAO 2013: E-ISBN 978-92-5-107929-4 (PDF)]. The review was based on an assessment of different biogas systems in China and Vietnam and some technical work done in Nepal. It also reviews scientific literature on use of bio-slurry as fertilizer in agriculture published since 1986. The review also notes that 60% of the biogas plants then commissioned in Vietnam did not make any use of the slurry. The review suggests that the bio-slurry is actually "Brown-Gold" (7).

Scientists at NDDB (3) describe development of 7 bio-products based on bio-slurry. The study reports how manure management value chain, can effectively double the farmer's income. The use of bio-slurry based Sudhan bio-products in agriculture has shown encouraging results with enhancement in the yield of different crops in the range of 14% to 36% percent over farmers' traditional practices under demonstration trials conducted on farmers' fields during kharif and rabi seasons of the year 2019-20. The average increase in the yield was reported to be around 28%. The study uses only 3 of the 7 products developed, namely Sudhan PROM, Sudhan Grade III, and Sudhan Root Guard. Other growth parameters like plant height, number of tillers, fruits, balls, or spikes per plant were also recorded to be better than the control. Crops considered in this study were rice, wheat, brinjal, chilli, tomato, papaya, cotton, castor and banana.

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Another study carried out by the same group over 2020-21 describes impact of gobargas slurry based organic products of Sudhan brand on crop yield (8). The study used two Sudhan products – Sudhan PROM, and Sudhan MRL. With sample size of 640 farmers (Out of 5000+ actual users) they studied the effect of these products on 35 crops. Yield improvement data is presented for 22crops, which ranges between 17% and 22% with an average of 19%.

Apart from the yield increase, there are reports showing better growth of crops in alkaline (9), saline soils (10), with the use of biogas bio-slurry. There are reports suggesting improvement in the fertility of calciferous soils with the use of bio-slurry (11), and improvement in the soil structure with combined use of bio-slurry and fly-ash (12). There are reports on mitigation of some fungal (13), viral and nematode (14) attacks on crops with the use of bio-slurry based products. If only nematode diseases are managed well using bio-slurry based products, farmers will save huge losses (20 - 60%) and costs on chemicals. There are also some reports on avoidance of some insect infestations with use of bio-slurry.

Such an exhaustive data on benefits of using slurry as a fertilizer in agriculture could lead to compulsive use or recommendation of the bio-slurry in the agriculture practices. However, this data only indicates what bio-slurry and slurry based bio-fertilizers can do. More work is needed on delivering the actual expected results to farmers via use of bio-slurry.

From a farmer perspective, it is important to know; when bio-slurry should be used, and for what purpose; how bio-slurry works, and what are the required peripheral factors; why will it benefit the crop, and what are the limits of its performance; and finally what are the expected economic benefits. For this repetitive, multi-location, multi-season performance studies with all soil and crop analysis data (at least with reference to minerals under consideration) is needed. This can lead to cause and effect hypotheses, and the product recipes used to prove the hypothesis, can then be productized. Objective evaluation, is needed before we can standardize the bio-slurry based products. An alternative approach could be to provide mechanistic analytical data of performance of the bio-slurry.

Further, by the time, the usage of the bio-slurry based products is about to get rationalize, we must have SOP for handling and processing of the bio-slurry. Backed by analytics for reproducible quality of the products, such studies will lead to products with defined expected results. The data on the assured economic benefits of use of bio-slurry based products must be unequivocally generated at commercial farmer fields.

Any ambiguity in use case interpretation, failure in standardization of the product/ method of application may prove disastrous for the ambitious promotion of bio-gas plants. As an example, let us consider the data presented in the book entitled "Bioslurry: A supreme Fertilizer" (6) which is referred above. The yield increase data in the book is very lucrative. However, this report is based on biogas programs of Hivos and SNV that are implemented in eight African countries. According to the same book, farmers in this region find it difficult to use the fertilizers (chemical) even at low level (<25 kg/ha), because the prices of these (nitrogenous chemical fertilizers) are high. Therefore, if this data is for farmers using very low levels of chemical fertilizers (which is not recorded in the research methodology), the said data may not be relevant at all to most of the India farmers.

There are a few research oriented articles that give analytical data of effects of using bio-slurry under various and defined test conditions. But these are not within the scope of the present discussion, where the greater concern is about reproducible benefits of using bio-slurry to commercial farmers. Essentially, there must be standardization of slurry handling, product formation, and cause-effect or mechanistic understanding of the working of the bio-slurry based products at commercial scale production, so that the bio-slurry based bio-fertilizers really become meaningful to the farmers.

# VII. TOWARDS STANDARDIZATION OF SLURRY HANDLING, AND PROCESSING

1. Characterization of Slurry: Slurry is so complex that its complete analysis will be prohibited by the cost. Also it is so unstable, that within the time required for analysis, it will have drastically changed its composition. Practically we can determine pH, EC, and relative solid %. For a given raw material composition, bio-gas plant design, and operating conditions; these three test parameters reasonably define slurry characteristics.

Apart from the analysis, certain practices would add – in multi-folds - to the actual performance of the products for which the farmer pays.

- Slurry must be taken for processing as soon as it leaves the bio-gas tank.
- Use of proper stabilizers of nutrients immediately on receiving the slurry is very important.
- Any adulteration with extraneous matter (rain water could be common incidental example) must be avoided.
- Slurry being processed must be stored away from direct sunlight, and under cool conditions.
- 2. Addition of Ingredients to Activate the Slurry for the Process: We have identified organic, inorganic additives and beneficial microbial cultures, which when added to the slurry, activate it for the further process. The recipe of the additives may have to be changed depending on the slurry quality and further process to be taken up. The addition of beneficial microbial cultures also effects stabilization of the slurry. Some process aspects of productization are covered under the Provisional Patent No: 201921035731 "Value added soil amendments from animal dung"
- **3.** Actual Separation of Solids and Liquid: There is ample literature available on tools and techniques of solid liquid separation from bio-slurry (15-17). We base our process on use of inorganic salt mixture, which tends to flocculate and raise up the slurry solids as a thick scum on the surface. The features of this process are as follows:
  - The composition of the salt mixture used constitutes 100% of crop nutrients.
  - The composition of the salt mixture can change as per the requirements of crops / soil conditions.
  - The salt mixture also stabilizes the volatile nitrogen to a great extent
  - The actual separation of solids and liquid can be done using a simple filter screen mesh at domestic plant level or using some machines at a large scale.

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• Solid –liquid separation: Provisional Patent: 201821042739 "Value added soil amendments from animal dung"

We have run this process with low cost tools at 100 - 1000 Ltr bio-slurry per day and at 5000 - 12000 Ltr bio-slurry per day using screw press. The low cost set up can be operated using manpower and mechanical tools only, and does not require electricity.

4. Addition of Biological Ingredients for Making Fully Active Product(S): Swasti Agro and Bioproducts Pvt Ltd has been working in the space of organic fertilizers and disease mitigation since 2011 (18-22). Swasti held NPOP standard 'organic certification' for its 8 Bioproducts which include 6 products from the table below (except PROM). Thus, functionality of these products, their active ingredients was all well documented and proven at the time of the certification. Except "Micronutrient Grade Products", which were already formulated in bio-slurry; remaining 5 products were being formulated in water. These products, formulated in water, when used in the field needed support of some products based on beneficial microbes in order to get the desired results.

We presented 7 distinct functional products by re-calibrating biological ingredients of our already certified organic products that were stabilized in the bio-slurry instead of water. The microbes from the slurry played role of the additional application of 'beneficial microbe'. Thus cost of separate application of beneficial microbes was eliminated.

While developing these bio-slurry based bio-products we had following challenges:

- The product formulation should be such that all the components are compatible to each other and form a uniform solution/ suspension.
- The product formulation should retain its activity for a considerable time say one year.
- The organic matter added to the formulation should not cause death of the microbes present in the formulation.
- The microbes present in the formulation should not get activated because of the organic matter added and start growing. This could result into gas formation leading to bulging and bursting of the air-tight container in which the formulation is stored. At least the biological activity of the product is definitely hampered.
- In order to make the product cost competent, we wanted to minimize the addition of any organic matter and rely more on the beneficial microbes from the slurry and at the same time did not want to compromise with the desired results on the crop.
- We wanted to liquidate large amount of bio-slurry in shortest possible time through the liquid products developed using bio-slurry.

The bio-slurry based bio-products that we defined based on purpose / use in agriculture, are described in the following table. Meeting all the ideal criteria for products was not possible with a single type of formulation. So we proposed three types of formulations as follows:

- Highly concentrated products with highest shelf life. Typical dose of application in the range of 250 ml 1000 ml per acre per application.
- High dosage of bio-slurry typically about 20 Ltr per acre per application, which is accompanied with specific bioactive organic additive in a separate bottle of about 250 ml. The organic additive to be mixed with the slurry at the time of application.
- High dosage of slurry typically up to 50 Ltr per acre per application, which is accompanied with organic food for the microbes in the slurry given in a separate bottle of about 1000 ml. The food and the bio-slurry is to be added to 200 L water, and kept aside for 18- 72 hrs to allow the microbial growth; and then applied to the field.

Based on the first two types of formulation, 7 functional products were developed and taken to the field. The third one is still at lab level. Distinct marketing strategies and business plans have been evolved for these three types of bio-products based on the bio-slurry.

Table 1: Different Bio-products developed by Swasti Agro based on Bio-slurry\*

Category	Product	Contents	Action
Nutrient Management	PROM (Solid)	Bio-slurry solids and Rock phosphate (3:1) fermented with beneficial microbes	Solubilize P biologically. Can replace chemical fertilizer (SSP)
	Micronutrient Grade Product	Bio-slurry liquid, salts, beneficial microbes	Increase availability of micro-nutrients
Disease Mitigation	Root-Guard	Modified chitosan, lactic acid, acetic acid, trace elements, fatty acids, Bio-slurry liquid	Improves root growth Protects roots from infections
	ICON 4	Soluble humate fraction, salts, micronutrients, fatty acid derivatives, bio-slurry liquid	Promotes plant and root growth.  Mitigates nematode infections
	Chito-Guard	Modified chitosan, lactic acid derivatives of salicylic acid, Bio-slurry liquid	Prevents entry of pathogens
Plant Growth Promotion	Wonder	Extracts of marine algae and microbes, Bio-slurry liquid	Promotes plant growth, photosynthesis, and vegetative growth
	LuxGro	Chito-oligosaccharides, proteinecious extract of microbes, Bio-slurry liquid	Protects vegetation and promotes flowering as well as fruit growth

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\*Some specific components used in these formulations are covered under a patent WO2018042311A1 "Chitosan derivative formulations for plant growth and building disease resistance"

5. Determination of Shelf Life of the Product: Shelf life of the bio-slurry based products mainly depends on the proper handling of bio-slurry and its quick stabilization. Within the prescribed shelf life, the slurry based product must confirm to (i) its biological activity as determined by the bioassay (ii) integrity of the chemical constitution (iii) retention of the microbial count up to minimum permissible level.

Since the slurry based products are new, standards and SOPs will have to be set with respect to each of these parameters.

# VIII. SOME NEW ASPECTS OF ANALYSIS FOR THE BIO-SLURRY BASED **PRODUCTS**

1. Microbial Analysis: 16S METAGENOME BASED TAXONOMIC PROFILING of 6 month old slurry based product (then called as GOBERDHAN) was carried out to understand microbes present in this product. The study read / sequenced 1.2x 10° mRNA data and concluded about the bacterial population of the formulation as follows.

 $1.38 \times 10^5$ **Total count of bacteria:**  $6.03 \times 10^4$ • Number of archae-bacteria (methanogens): 92

• Number of archae-bacterial species:

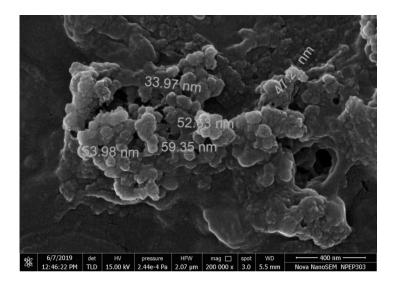
 $7.81 \times 10^4$ • Number of eubacteria: **Number of archae-bacterial species:** 1404

When we actually determined the microbial count of the samples of the product from the same batch monthly, over one year period; we recorded no significant change in the TVC. However, we could isolate only 5 - 32 types of distinct colonies by the morphology from distinct product samples. All these must be aerobic non-methanogenic bacteria.

2. Formation of Special Composite Structures of the Added Nutrients: P. Suganya, and P.U. Mahalingam have established simple method for synthesizing iron nano- particles oxide using vermin-wash from earthworm compost. They describe initial characterization of these nano-particle using UV-Visible spectral analysis (23).

Taking clues from this work, we used UV-Visible spectral analysis to define method(s) for addition and reaction of various minerals with different samples of bioslurry. The nano-particles of minerals are formed in the bio-slurry, as if it is a natural occurrence, unless some simple instructions about the process are not followed.

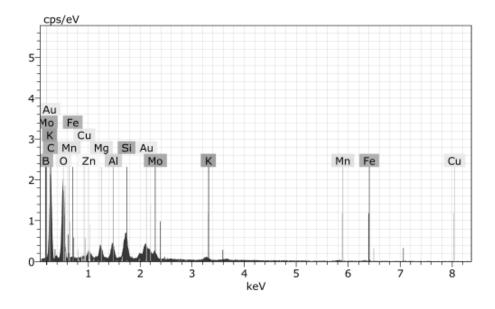
Micro-nutrient minerals added in small to quantity (typically one tenth or even less than their contents in the conventional micro-nutrient fertilizer grades) to the product formulation(s) get organized in special composite structures. These structures have 30 – 50 nm diameter as detected in electron microscopy (Figure 1).



**Figure 1:** Nano-particles detected by electron microscopy show composite structures of about 30 nm size

The XRD analysis of such composites revealed that these are heterogeneous chemically. Each composite contains different elements at different concentrations. We can improve preference to specific element being included in these composites by making small changes in the sequence/ process/ steps of addition.

The XRD data also revealed (Figure 2) that some of the essential nutrients like Si, K, Mg, Au are present in the composite structures, which are not added chemically to the reaction, and may be coming from the slurry composition.



**Figure 2:** Identification of minerals present in the nano-particle composites using XRD technique

# IX.INNOVATIVE FINDINGS ABOUT PERFORMANCE OF BIO-SLURRY BASED PRODUCTS

Bio-slurry based products developed by the method that generates nano-particle composites, and using less than 10% of the mineral contents with reference to those described in FCO have been used in performance assay. Total viable count of these formulations was also recorded.

- 1. Uptake of Mineral Nutrients: The concentrations of the nutrients absorbed in leaf was found to be 5 − 1000 times better when bio-slurry based nutrients were used over its conventional chemical counterpart. These were tested under controlled conditions and analyzed by third party NABL accredited lab. Different elements preferred distinct modes of application viz. foliar spray or soil drenching for enhanced uptake. In an experiment done on fenugreek, Molybdenum contents of the leaf were found to be enhanced by 150 folds over control where the Molybdenum applied to the crop was almost 10 times higher.
- 2. Performance Assays for Mitigation of Diseases Using Bio-Slurry: At Swasti Agro and Bioproducts Pvt Ltd, we had a DSIR approved R&D laboratory; that was dedicated for research on mitigation of plant diseases. We developed methods -based on internationally approved research work- that could detect capacities of microbial cultures or their metabolites to mitigate diseases of crops in various ways. Significance of three such tests deployed in analysis of bio-slurry based products is described below.
- **3. Pathogen Binding Assay:** As a first step of interaction, any pathogen first binds the surface of the plant. It forms micro-colony, biofilm; and then at right stage all the pathogens in this biofilm signal each other to invade / attack the plant. In this assay, the pathogen is mixed with desired microbe or its metabolites; and the reduction in the capacity of the pathogen to bind the plant surface is recorded as inhibition in "pathogen binding assay"

When bio-slurry based products were tested by this method against pathogens like Xanthomonas, and Fusarium, significant inhibition of binding was observed. Microbes from the bio-slurry, their metabolites with and without nano-particles, and metabolites free from microbes were tested separately. All these four fractions showed significant inhibition in binding activity of pathogens.

**4. Nematode Inhibition Assay:** Nematode is a very common problem worldwide. It affects all crops. According to a published finding of 2007 (24), India loses over Rs. 20,000 cr. worth produce every year because of nematode. The chemical nematicides work only as contact poison and therefore have a limited utility. According to a report on okra (25), chemical treatment(s) could avoid only 20 – 40% yield loss. The losses due to nematodes in India were estimated to be between 17 and 33% (Rs 16000 – 80,000 per acre) during an exercise conducted under AICRP-nematode program.

We have developed a bioassay using *Caenorhabditis elegans* – a harmless culturable nematode, and proven the activity of nematode inhibition for our patented chitosan derivatives. Bio-slurry has an anti-nematode activity displayed by the same assay. Boosting it with low levels of chitosan derivative helped standardize the products

and offer a decision- matrix for farmers (based on one in-field detection of nematodes) to use right method / bio-slurry based product.

**5. Fungal Spore Inhibition Assay:** We have developed two types of bioassays using spores of pathogenic fungi grown in the laboratory. One bioassay used microscopic detection of germination recorded non-germinated / killed spores. In the same assay, reduction in the number of intact spores gives a score of spores degraded during incubation period. Thus number of spore not germinated + number of spores degraded = total number of eliminated fungal spores.

The other assay is based on determination of total viable count by plating out. The first one is rapid, accurate, and also provides mechanistic insights.

Using the microscopic assay, when bio-slurry was used as it is, it could eliminate 91% spores of Fusarium sp. However, the diluted samples were ineffective. When the bio-slurry products were formulated using low quantities of modified chitosan, enzymatic activity of chitosanase was enhanced, leading to increase in the spore degradation and effective fungal control even at dilutions as high as 200 folds.

#### X. DISCUSSION

Innovative technologies developed at Swasti Agro over last 12 years for production of biological material and its use in agriculture for plant growth promotion, nutrient and disease management have been integrated with the bio-gas based bio-slurry. This offers many techno-commercial advantages over formulation of similar products in water, and / or using bio-slurry without any further processing. The productization process has been smoothly run at operational scale of 100 Ltr, 1000 Ltr and 10,000 Ltr per day using distinct set ups which cost about Rs 10,000, 2.5 Lakh, and 100 Lakh respectively.

Production of 6 distinct liquid products is important for utilization of complete liquid portion (about 70% vol/vol) of the total slurry. A single household biogas plant of 2 m<sup>3</sup> produces 2000 – 3000 Ltr slurry per month (100 Ltr per day). Such a plant can cater bioslurry based bioproducts to 30 – 100 acres of fields throughout the year. Thus larger plants that process 1000 and 10,000 Ltr slurry per day will respectively need 1000 and 10,000 acres of farms using the bio-slurry based products on regular basis and throughout the year. Therefore marketing of these products must be done on a very large scale. Unless a large number of farmers are happy using different products manufactured from bio-slurry, all bioproducts based on bio-slurry can't be liquidated.

The improvement in the performance of the bio-slurry is possible because of the specific processes, additions, and stabilization; and provides scope for tremendous value addition. A farmer having a 2 m³ domestic biogas plant can get 3000 Ltr slurry per month. The slurry can be processed at the farmer end to obtain minimum of about 2000 Ltr slurry liquid and about 500 kg of PROM, i.e. solid fertilizer. When the quality additive material is supplied free to the farmer, the quality finished material can yield Rs 14,000 per month at a rate of about Rs 5 to 6 per kg or Ltr of the pre-processed material. The consumable material required for processing can be delivered to the farmer, and pre-processed material lifted after the required quality checks on monthly basis. Thus we foresee great income potential for the

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farmers, substitution of huge amount of chemicals, reduction in use of toxic chemicals as a result of right use of these products.

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