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Final Report of Minor Research Grant

Microarray Analysis of Algae for Cellulose, Hemicellulose and Lignin Content and Study on Algae as Promising Supplement for Paper Pulp

Ref No: MRP-4904/14/ (SERO-UGC) Dated March 2014.
Comcode: TNBA007

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INTRODUCTION

Paper is one of the major products of the forestry industry and has a wide application in the society. They are not only used for publishing industry and writing but also on specialty papers, cardboards and brown papers.Wood pulp is the fibrous material that results when wood is separated into its constituent fibers by chemical or mechanical means. Waste paper is composed of previously discarded paper or paperboard products. Both contain cellulose fiber that can be subsequently combined with other inputs to manufacture paper, paperboard, or other wood-fiber-based products.

The major components of wood are cellulose (70-80 %) and lignin (20-30 %). Lignin is the material that binds cellulose fibers together.Wood pulp results when wood is separated into its constituent fibers by either chemical or mechanical etcbefore using it for paper production. One of the major steps in paper production is that of removal of lignin which could hamper or make the whole process fruitless. Thus carefully treating the pulp to remove the lignin is an important parameter in the paper production (Fred Forstall, 2002).

The current paper industry is dominated by the usual developed nations in North America, Northern Europe, East Asia and Australia. United States was the market leader but China in 2009 overtook them and became the leader in this environmental degradation process (De Sisti, Mike, 2012). China is the founder nation of paper making as well as the current market leaders in paper industry. But the pulp industry is one among the few industries in China which is facing a shortage of the raw material - pulp. They have been forced to import this from the other nations in order to maintain the trade balances (ZhongZhaung*et al*., 2006).

In the Indian scenoria, the paper industry is considered as one of the most polluting sectors within the economy and has been a topic of discussion in both local and global environment discussions. There has been a consistent wave of improvement throughout the ages to bring out a cleaner and more efficient technology in the manufacturing sector in order to bring parallel economic, environmental and social development objectives (Katja Schumacher and JayantSathaye, 1999).

Like China, domestic wood fiber supply is constrained in Japan, and most of Japan’s production goes towards meeting domestic demand. Japan’s industry has become adept at using recycled fiber, especially in higher, value-added grades of paper. Indonesia

has a large wood fiber base, and pulp and paper production was an integral part of its national land and forest resourcemanagement plan.

There has been a consistent rise in research related to alternative pulp source which are rich in fibers as well as lacks lignin. This is mainly seen in countries which has a massive shortage of the wood fibers. The shortage of wood fibers along with its massive use for paper and other industrial process has increased its rate in the market (Mudit Chandra, 1998). This is the main reason for china being the leading nation in paper industry related research. The Chinese industry has a huge gap between demand and supply of raw material and finished product too (Michel Valois *et al*., 2012).

Many other countries are also looking for nonwood plant fibers for papermaking that is easily available in their region. Some of these countries have pulp production mainly based on nonwood plant fibers resources based on the availability. China and India - depend on nonwood plant fibers for more than 50% of pulp production (Atchison, 1992 b). The economic advantage in many developing countries is the presence of nonwood resources, a growing domestic market for paper, reasonable labor costs, and absence of wood raw materials (MacLeod, 1988).

Some of the nonwoodfibers are agricultural residues such as Sugarcane bagasse (*Saccharumofficinarum*), Corn stalks (*Zea mays*), Cotton stalks (*Goossypium*), Rice straw (*Oryzasativa*), Wheat straw (*Triticumaestivum*), Cereal straw then some of the naturally growing plants such as Bamboo (*Dendrocalamusstrictus*), Esparto (*Stipatenacissima*), Reeds (*Phragmitescommunis*Trinius), Sabai grass (*Eulaliopsisbinata*), Papyrus (*Cyperus papyrus*) and non-wood crops such as BastFibers, Jute (*Corchoruscapsularis*), Ramie (*Boehmerianivea*), Sunn Hemp (*Crotalaria juncea*), Hemp (*Cannabis sativa*), Kenaf (*Hibiscus cannabinus*), Abaca (Manila hemp) (*Musa textilis*), Sisal (*Agave sisalana*), Flax tow (*Linumusitatissimum*), Old ropes or rags made from these fibers, Leaf Fibers, Abaca (Manila hemp) (*Musa textilis*), Sisal (*Agave sisalana*), Seed Hair Fibers, Cotton fibers, Cotton linters, Cotton rags, Textile wastes of various types (Mudit Chandra, 1998).

The research on use of algae especially red algae in paper making was initially done in China. The reason for using red algae is mainly because of the presence of less lignin content in them (Yung-Bum Seo*et al.*, 2009). Algae is preferable for paper making because of the presence of cellulose, hemicelluloses and fiber content in them which makes it

suitable alternate and supplement for pulp. The use of algae was also tested on par with that of lemon peels for making tissue paper. This provides base for use of microalgae as a source for pulp preparation (C.Ververis *et al*., 2007).

There are other products too which are derived from Microalgae and having wide application in industrial and therapeutic field. They have various pigments and carotenoids such as β-carotene, astaxanthin, lutein, zeaxanthin, canthaxanthin, chlorophyll, phycocyanin, phycoerythrin, fucoxanthin. Substances like poly unsaturated fatty acids could be derived from them. Production of vitamins such as A, B1, B6, B12, C, E, biotin, riboflavin, nicotinic acid, pantothenate, folic acid. They also have antioxidants which could be extracted like Catalases, polyphenols, superoxide dismutase, tocopherols. Some of the other substances are Antimicobial, antifungal, antiviral agents, toxins, aminoacids, proteins, sterols, MAAs for light protection. Thus cultivation of microalgae is considered to be profitable business as it is wasteless, ecologically pure, energy and resource saving process (Indira Priyadarshani and BiswajitRath, 2012).

Marine algae also known as seaweeds commonly had industrial application. The discovery of industrial application began in the early 19th century when the hydrocolloid was found by Charles Stanford. Since then there was a widespread use of seaweeds in the production of different hydrocolloids (BernaKılınç*et al*., 2013). Seaweeds have wide range of therapeutic activities both internally as well externally. They are known as one of the best sources of dietary essential minerals. It is said that seaweeds are 20-50% of mineral (Kazutosi, 2002). Some of the most common and abundant minerals found are potassium, sodium, calcium, magnesium, zinc, copper, chloride, sulfur, phosphorous, vanadium, cobalt, manganese, selenium, bromine, iodine, arsenic, iron, and fluorine(Dr. Ryan Drum, 2003).

Marine algae contain large amounts of polysaccharides, notably cell wall structural, but alsomycopolysaccharides and storage polysaccharides (Kumar et al. 2008b; Murata and Nakazoe 2001). Polysaccharides are polymers of simple sugars (monosaccharides) linked together by glycosidic bonds, and they have numerous commercial applications in products such as stabilisers, thickeners, emulsifiers, food, feed, beverages etc. (McHugh 1987; Tseng2001; Bixler and Porse, 2010). The total polysaccharide concentrations in the seaweed species of interest range from 4-76 % of the dry weight The highest contents are

found in species such as *Ascophyllum*, *Porphyra*and *Palmaria*, however, green seaweed species such as *Ulva* also have a high content, up to 65 % of dry weight.

Seaweeds are low in calories from a nutritional perspective. The lipid content is low andeven though the carbohydrate content is high, most of this is dietary fibres and not taken upby the human body. However, dietary fibres are good for human health as they make anexcellent intestinal environment (Holt and Kraan, 2011).The seaweeds also contain phycopolymers which are also known as mucopolysaccharides. These mucopolysaccharides constitute about 20-45% of their total weight. Algin and Fucoidan are sulfatedgycans and carrageenan. These have various properties such as heavy metal detoxifying agents, anti inflammatory agents, constipation, soar throat etc. Thus they have a wide range of application in surgery, anti viral agents, respiratory illness, erectile dysfunction etc. They also are sources for hormones, essential fats and vitamins. Some of the hormones present in seaweeds are melatonin, thyroid hormones, di-iodothyronine (DIT) and thyroxine. Essential fats and vitamins that are present in seaweeds are omega 3-fatty acids, vitamin B, B12, A and C (Dr. Ryan Drum, 2003).

The cell wall polysaccharides of seaweeds constitute cellulose, hemicelluloses and some neutral polysaccharides which supports for the use of them for pulp production. Even they have a low lignin content which is necessary for pulp preparation. The presence of fiber in them assures for strength of the final product (Stefan Kraan, 2012). The presence of these attributes is due to their close relevance to plants (Debashish Bhattacharya and Linda Medlin, 1998).

One of the most obvious and arresting characteristic of the algae is their colour. In general, each phylum has its own particular combination of pigments and an individual colour. Aside chlorophylls, as the primary photosynthetic pigment, microalgae also form various accessory or secondary pigments, such as phycobiliproteins and a wide range of carotenoids. These natural pigments are able to improve the efficiency of light energy utilization of the algae and protect them against solar radiation and related effects. Their function as antioxidants in the plant shows interesting parallels with their potential role as antioxidants in foods and humans (Van den Berg *et al*., 2000). Therefore, microalgae are recognized as an excellent source of natural colorants and nutraceuticals and it is expected

they will surpass synthetics as well as other natural sources due to their sustainability of production and renewable nature (Dufossé*et al*., 2005).

All algae contain one or more type of chlorophyll: chlorophyll-*a* is the primary photosynthetic pigment in all algae and is the only chlorophyll in cyanobacteria (blue-green algae) and rhodophyta. Like all higher plants, chlorophyta and euglenophyta contain chlorophyll-*b* as well; chlorophylls -*c*, -*d* and –*e* can be found in several marine algae and fresh-water diatoms. Chlorophylls amounts are usually about 0.5-1.5% of dry weight (Becker, 1994).In the algae the carotenoids seem to function primarily as photoprotectiveagents and asaccessory light harvesting pigment, thereby protecting the photosynthetic apparatus against photo damage (Ben-Amotz*et al*., 1987). They also play a role in phototropism and phototaxis (Borowitzka, 1988). Some microalgae can undergo a carotenogenesis process, in response to various environmental and cultural stresses (e.g. light, temperature, salts, nutrients), where the alga stops growth and changes dramatically its carotenoid metabolism, accumulating secondary carotenoids as an adaptation to severe environments (Bhosale, 2004).

Physical and optical properties of paper: Basis weight, bulk, brusting strength, tearing strength, tensil strength, breaking length, smoothness, porosity, brightness. Basis weight is the "weight of paper per unit area" and it is expressed as grams/sq. meter. Tear is the force required to tear a single sheet of paper through a specified distance and is expressed as Tear Factor.

The papers produced from algae, both macro as well as microalgae has less brightness due to the presence of chlorophyll. The paper produced frommacroalgae had a brightness of over 80% surprisingly, high bekk smoothness and opacity which supports for the high value printing grade papers (Yung-Bum Seo*et al*., 2010). The paper produced from microalgae has less brightness and decreased tearing resistance but they have a high mechanical strength (C. Ververis*et al*., 2007).

Paper is an important commodity and will see an exponential rise in the years to come. Paper is generally defined as a single ply, flat material, varying in density and material content according to end use. Paper is one of the core industries and is linked to the basic human needs. Paper is the pre-requisite for education and literacy and its use is an index of advancement in these two fields as well as the overall well being of the society.

Growth in pulp and paper production entails massive felling of trees, which in turn leads to deforestation. Increasing competition for wood supplies coupled with gradually rising costs of wood have generated renewed interest in the use of non wood plant fibers for papermaking. Paper production from non wood fibers is particularly significant in China and India where there are a large number of very small mills (Sarah Roberts, 1996).

Many of the non-wood fibers are similar to the short fiber hardwoods, while others are so long that they must be shortened to optimize their papermaking value. In general, the diameter of the non-wood fiber is small, resulting in lower coarseness from these pulps. These fiber dimensions provide an idea of the potential usefulness of these pulps in pulp and papermaking. The sources of fiber are agro residues and forest which might not be enough for future needs of paper production. The forest and agro residue need to undergo a lot of pre-processing for lignin removal before the extraction is achieved. The widely used chemical process has been shown to have just about 30-50% effectiveness of fiber extraction. Various different alternative sources such have been studied widely, one such source is algae.

Photo synthetic aquatic species i.e., micro and macro algae and fresh or salt water plants are used for papermaking. Photosynthetic aquatic species having cellulosic and fibrous characteristics and that is necessary for paper production. Several species of algae are reported to possess cellulose in quantities greater than 10% of total dry weight (Knoshoug P *et al*., 2012). With many distinctive properties algae seems as a good fit for being the alternative source. Previously, people have worked on using red algae, suspended micro-algae for fiber extraction and have observed good yield and productivity. There are tons of algae and tidal waste removed each year on our shores and its scarcity of lignin, these residues may be an adequate source of cellulose. In papermaking, lignin must be removed and separated from the cellulose fibers; this step is responsible of the generation of hazardous contaminants and an elevated consumption of energy (Jimenez L *et al*., 2005). Previous studies have characterized that the algae contain very low quantities of lignin and high fiber contents. The amount of solvent substance, lignin, hemicellulose in dried algal pulp is estimated. The algae have low lignin like compounds and solvent soluble substance content, which supposed an enormous advantage over the current cellulose extraction methods as it eliminates the need of pre-treatment, cooking and bleaching stages.

Therefore, the application of extremely toxic reagents used nowadays is not necessary for application of algae as supplement for pulp material (Kiran *et al*., 1980, Chao *et al*., 2000). Hence, for development of paper pulp, most energy is wasted on lignin removal. Exploitation of well bonded algal pulp can yield paper that requires no artificial treatments for lignin removal. It was claimed that paper production from red sea weeds algae (Tronchin EM *et al., 2002)* compared to wood pulp, takes shorter time; lower cooking temperature and minimize chemical usage. Compared to wood fibers, algal fibers are finer, more uniform in length, smoother, also have absorbent properties and may not require fillers. Cellulose from some genera of filamentous green algae exhibit particularly high degree of crystallinity and exhibit preponderance of I-alpha; cellulose as opposed to I-beta cellulose, making them more thermodynamically reactive in comparison to cellulose derived from woody or plant biomass.

Ubiquitous distribution and fast growth of algae mark their easy availability as natural resources and possibility of harvesting all year round cuts down the costs involved in farm land cultivation or resource import or transport (Kaushik N *et al.,* 2014). Unwanted blooms of algae throughout the world are not entirely without a positive side. Algae as a raw material for paper making are an innovative solution to Global environmental issues dealing with deforestation and global warming. Value added products from algae also hold much promise in generating alternative sources of livelihood generation from the economic point of view. Carbon dioxide sequestration, removal and productive utilization of fouling biomass from paddy fields and alternative income generation to the rural people are certain other aspects that may make additional input in bio-remediating environment by direct or indirect means (Piyali Mukherjee and Jai Prakash Keshri, 2018). The significance of the research is to use organic materials such as algal biomass as supplementing for paper manufacturing. Supplementing Algae in pulp material increases the mechanical strength of paper due to the binding property of protein and chitin of algal cells. On the other hand mixing of algal biomass with conventional paper pulp seems a more promising method for exploiting algae from eutrophic water.

The present paper has been attempted to make an extensive search for suitable algal source to be used in paper pulp supplement. Different algal samples were collected from freshwater, marine and effluent sources to evaluate their biochemical characteristics and

cellulose, hemicellulose contents which are the prerequisites for paper making raw materials were estimated in various algae. Then algae were also assayed for their pigment production ability. Hand made papers was tried of algae from effluent sources and their quality were analysed in Seshasayee Paper Mills limited. Thus the present research gives a new dimension to application of algae and waste algal resources.

AIM OF RESEARCH

The main aim of this research is to develop the applications of algae for supplementing pulp material in papermaking and to increase the mechanical strength of paper due to the binding property of protein and chitin of algal cells.

OBJECTIVES OF THE STUDY:

1. Collection of microalgae and macroalgae samples of large quantities
   - Collection of Microalgae sample from fresh water bodies (Sirumugai, Bhavani, Siruvani etc.,), Macroalage (Marine algae) from Mandapam and Tuticorin.coastal region of Kanyakumari and effluent treatment plants.
2. Morphological identification of both microalgae and macroalgae samples.
3. Cultivation of Microalgae in laboratory using BG 11 medium.
4. Morphological characterization of the cultivated Microalgae.
5. To identify, and perform characterisation, cultivation and optimisation of algal sample.
6. Estimation of reducing sugar, cellulose, hemicelluloses, lignin, ash, lipid contents.
7. Preparation of paper using Microalgae in the laboratory.
   - Paper preparation using different concentration of Algae (10, 20, 30, 40 and 50%) by varying the concentration of pulp accordingly.
8. Analysis the quality of algae supplemented paper by different tests.
   - To process the pulp for papermaking and assess the quality of paper material
   - To apply the algal paper pulp in packaging material

REVIEW OF LITERATURE

The word ‘algae’ originates from the Latin word which means seaweeds. Algae represent a large group of different organisms from different phylogenetic groups, representing many taxonomic divisions.In general algae can be referred to as plant-like organisms that are usually photosynthetic and aquatic, but do not have true roots, stems, leaves, vascular tissue and have simple reproductive structures. It consists of both micro and macro organisms. They are distributed worldwide in the sea, in freshwater and in wastewater. They also include organism from Prokaryotes (cells lacking membrane bound nucleus) as well as Eukaryotes (cells having membrane bound nucleus and organelles) (Tuchman, 1996). Macro algae fall into three basic groups, Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae). Microalgae consists of the prokaryotic Cyanobacteria which are either found as free living or found in symbiotic association with fungi and also Eukaryotic forms. Algae have been estimated to have more than one million species which are grouped in to 15 phyla and 54 classes (Michael D, Guiry 2012).

Microalgae are unicellular photosynthetic organisms that are present in almost all eco-systems on Earth. There are numerous opportunities of using microalgae (Energy, Nutraceutical Production, Biomaterial production etc.,). There are many advantages of micro-algal biomass production, for instance they are easy to cultivate and have very high growth rates, microalgae can also grow in water that is unsuitable for human consumption, microalgae can absorb carbon-dioxide from the atmosphere through photosynthetic process. (Mata, Martine and Caetano 2010). Microalgaeis found in freshwater,waste water as well as sea water environments whereas the macroalgae is found only in marine and brackish waters (Barsanti *et al.,* 2006). Marine algae contain large amounts of polysaccharides, notably cell wall structural, but also mycopolysaccharides and storage polysaccharides (Kumar et al. 2008b; Murata and Nakazoe 2001).

Algae are primary producers and occupy the top most position in the food chain along with plants. Thus they play a very important role in nutrient recycling on par with plants. This attribute is because of the presence of photosynthetic pigments such as

chlorophyll and carotenoids (Bold & Wynne 1985, Van den Hock *et al.*,). Seaweeds are low in calories from a nutritional perspective. The lipid content is low and even though the carbohydrate content is high, most of this is dietary fibres and not taken up by the human body. (Holt and Kraan, 2011).

Some of the microalgae such as Diatoms and Dinoflagellates contain other pigments like xanthophylls which is similar in structure to carotenes. These organisms appear in yellow to brown and yellow to red in colour (Barsanti *et al.*, 2006). Algae constitute a polyphyletic group since they do not include a common ancestor, and although their plastids seem to have a single origin, from cyanobacteria, they were acquired in different ways. Green algae are examples of algae that have primary chloroplasts derived from endosymbiotic cyanobacteria. Diatoms are examples of algae with secondary chloroplasts derived from an endosymbiotic red alga. (J.D. Palmer, D.E. Soltis, M.W. Chase,2004).

The different mode of nutrition exhibited by them such as autotrophy, heterotrophy and mixotrophy.Most algal groups should be considered photoautotrophs, that is, depending entirely on their photosynthetic apparatus for their metabolic necessities, using sunlight as the source of energy, and CO_2 as the carbon source to produce carbohydrate and adenosine triphosphate. Most algal divisions contain colorless heterotrophic species that can obtain organic carbon from the external environment, either by taking up dissolved substance (osmotrophy) or by engulfing bacteria and other cells such as particulate prey (phagotropy). Algae use a complex spectrum of nutritional strategies, combining photoautotrophy and heterotrophy. This ability is referred to as mixotrophy (Laura Barsanti, Paolo Gualtieri).

PHYSICAL AND ECOLOGICAL FEATURES OF ALGAE

Filamentous forms have cells arranged in chains like strings of beads. Some filaments (e.g., *spirogyra*) are unbranched, whereas others (e.g., *Stigeoclonium*) are branched and bushlike. In many red algae (e.g., *Palmaria*), numerous adjacent filaments joined laterally create the gross morphological form of the alga. Coenocytic forms of algae grow to large sizes without forming distinct cells. Coenocytic algae are essentially unicellular, multinucleated algae in which the protoplasm (cytoplasmic and nuclear content of a cell) is not subdivided by cell walls. The green seaweed *Codium* has been called dead-

man's-fingers. Some algae have flagella and swim through the water. These flagellates range from single cells, such as *Ochromonas*, to colonial organisms with thousands of cells, such as *Volvox*.

Algae form organic food molecules from carbon dioxide and water through the process of photosynthesis, in which they capture energy from sunlight. Similar to land plants, algae are at the base of the food chain, and the existence of nonphotosynthetic organisms is dependent upon the presence of photosynthetic organisms.

The cell walls of many types of seaweed contain phycocolloids (algal colloids) that can be extracted by hot water. The three major phycocolloids are alginates, agars, and carrageenans. Alginates are extracted primarily from brown seaweeds, and agar and carrageenan are extracted from red seaweeds. These phycocolloids are polymers of chemically modified sugar molecules, such as galactose in agars and carrageenans, or organic acids, such as mannuronic acid and glucuronic acid in alginates. Most phycocolloids can be safely consumed by humans and other animals, and many are used in a wide variety of prepared foods, such as "ready-mix" cakes, "instant" puddings and pie fillings, and artificial dairy toppings.

PIGMENTS PRODUCED FROM ALGAE

One of the most obvious and arresting characteristic of the algae is their colour. Ingeneral, each phylum has its own particular combination of pigments and an individual colour. Aside chlorophylls, as the primary photosynthetic pigment, microalgae also formvarious accessory or secondary pigments, such as phycobiliproteins and a wide range of carotenoids. These natural pigments are able to improve the efficiency of light energy utilization of the algae and protect them against solar radiation and related effects. Their function as antioxidants in the plant shows interesting parallels with their potential role as antioxidants in foods and humans (Van den Berg *et al*., 2000). Therefore, microalgae are recognized as an excellent source of natural colorants and nutraceuticals and it is expected they will surpass synthetics as well as other natural sources due to their sustainability of production and renewable nature (Dufossé*et al*., 2005).

Chlorophylls

All algae contain one or more type of chlorophyll: chlorophyll-*a* is the primaryphotosynthetic pigment in all algae and is the only chlorophyll in cyanobacteria (blue-green algae) and rhodophyta. Like all higher plants, chlorophyta and euglenophytacontain chlorophyll-*b* as well; chlorophylls -*c*, -*d* and –*e* can be found in several marine algae and fresh-water diatoms. Chlorophylls amounts are usually about 0.5-1.5% of dry weight (Becker, 1994).

Apart from their use as food and pharmaceutical colorants, chlorophyll derivatives can exhibit health promoting activities. These compounds have been traditionally used in medicine due to its wound healing and anti-inflammatory properties as well as control of calcium oxalate crystals and internal deodorization (Ferruzi and Blakeslee, 2007).

Carotenoid

Carotenoids are naturally occurring pigments that are responsible for the different colours of fruits, vegetables and other plants (Ben-Amotz and Fishler, 1998). Carotenoids are usually yellow to red, isoprenoidpolyene pigments derived from lycopene. They are synthesized *de novo* by photosynthetic organisms and some other microorganisms (Borowitzka, 1988). In animals the carotenoids ingested in the diet are accumulated and/or metabolized by the organism, being present in meat, eggs, fish skin (trout, salmon), in the carapace of Crustacea (shrimp, lobster, Antartic krill, crawfish), and in the subcutaneous fat, the skin, the egg yolks, the liver, the integuments, and in the feathers of birds (poultry) (Breithaupt, 2007).

In the algae the carotenoids seem to function primarily as photoprotective agents and as accessory light harvesting pigment, thereby protecting the photosynthetic apparatus againstphoto damage (Ben-Amotz*et al*., 1987). They also play a role in phototropism and phototaxis(Borowitzka, 1988). Some microalgae can undergo a carotenogenesis process, in response to various environmental and cultural stresses (e.g. light, temperature, salts, nutrients), where the alga stops growth and changes dramatically its carotenoid metabolism, accumulating secondary carotenoids as an adaptation to severe environments (Bhosale, 2004).

The consumption of a diet rich in carotenoids has been epidemiologically correlated witha lower risk for several diseases particularly those in which free radicals are thought to

play a role in initiation, such as arteriosclerosis, cataracts, age-related macular degeneration, multiple sclerosis and cancer (Stahl and Sies, 2005; Tapiero et al., 2004). However, unexpected results from intervention studies (ATBC, 1994; Omenn*et al*., 1996) with β- carotene suggest that the threshold between the beneficial and adverse effects of some carotenoids is low and provides a strong stimulus to further understanding the functional effects of specific carotenoids (Van den Berg *et al*., 2000). The main carotenoids produced by microalgae are b-carotene from *Dunaliellasalina* and astaxanthin from *Haematococcus pluvialis*.β-carotene serves as an essential nutrient and has high demand in the market as a natural food colouring agent, as an additive to cosmetics and also as a health food (Raja *et al*., 2007).

Phycobiliproteins

Besides chlorophyll and carotenoid lipophilic pigments, Cyanobacteria (blue-greenalgae), Rhodophyta (red algae) and Cryptomonads algae contain phycobiliproteins, deep colored water-soluble fluorescent pigments, which are major components of a complex assemblage of photosynthetic light-harvesting antenna pigments - the phycobilisomes (Glazer,1994). Phycobiliproteins are formed by a protein backbone covalently linked to tetrapyrrolechromophoric prosthetic groups, named phycobilins. The main natural resources of phycobiliproteins are the cyanobacterium *Spirulina* (*Arthrospira*) for phycocyanin (blue) and the rhodophyte *Porphyridium* for phycoerythrin (red). They are extensively used for fluorescence applications, as highly sensitive fluorescence and for labelling antibodies used in multicolour immunofluorescence or fluorescence-activated cell-sorteranalysis (Becker, 1994).

APPLICATION OF ALGAE

Algae are by far the most abundant primary producers, although some can be mixotrophy or heterotropic. Algae are cultivated and used in nutrition worldwide. They are an important source of vitamin, minerals, proteins, polyunsaturated fatty acids, antioxidants, etc (Dajana J. Kovacl, 2006). They have been used in animal in human diets sice very early times filamentous algae are usually considered as ‘macrophytes’ since they

often form floating masses that can be easily harvested, although many consist of microscopic, individual filamentous of algal cells. Microalgal biotechnology only really began to develop in the middle of the last century but it has numerous commercial applications. Algal products can be used to enhance the nutritional value of food and animal feed owing to their chemical composition; they play a crucial role in aquaculture.

There is several utilization of algae or microalgae by which we can short out the environment problems like problem of global warming is CO_2, can be used reduce many heavy or toxic metals form waste water through a process called phycoremediation, can be used as biofuel which can be further processed by transesterification process to get the biodiesel which has the properties same as the our conventional diesel, they can also be used for the many cosmetics, food and many use in the field of pharmaceuticals (Vivek Prakash Pankaj, 2013). Microalgae can also be used to enhance the nutritional value of food and animal feed owing to their chemical composition, also play a crucial role in aquaculture, used as single-cell proteins, also in poultry and pharmaceutical industries due to presence of different useful compounds (Indira Priyadarshani and BiswajitRath, 2012).

Harvesting or aquacultures of marine algae are seaweeds is an extensive global industry. This seaweed industry provides a wide variety of products that have an estimated total annual production value of about US$ 6 billion (Stefan Kraan, 2012). Macroalgae are an important resource as food and shelter for a large range of fish, shellfish, and other invertebrate species, and they often act as nurseries for juvenile fish. As drift, seaweeds are a vital food source for many beach invertebrates and, when rotting on the sand, they return vital nutrients back into the beach ecosystem (Alan Millar, 2009). Seaweed are crucial primary producer in oceanic aquatic food webs. They are rich both in minerals and essential trace elements, and raw materials for the pharmaceuticals and cosmetics industry. Seaweed is a very versatile product widely used for food in direct human consumption (BernaKilinc*et al*., 2013). Seaweeds are also used to produce hydrocolloids; alginate, agar and carrageenan, which are used as thickening and gelling agents. Toda, approximately 1 million tonnes of wet seaweed are harvested and extracted to produce about 55,000 tonnes of hydrocolloids, valued at almost US $ 600million (McHug, 2003).

Waste Water Treatment:

Use microalgae for wastewater treatment are an old idea, and several researchers have developed techniques for exploiting the algae's fast growth and nutrient removal capacity. The nutrient removal is basically an effect of assimilation of nutrients as the algae grow, but other nutrient stripping phenomena also occur, e.g. ammonia volatilisation and phosphorus precipitation as a result of the high pH induced by the algae (Hammouda *et al*., 1994). Apart from tertiary treatment, microalgae may provide heterotrophs in secondary treatment with oxygen, and can also be used to absorb e.g. metals from mine wastewater. The increase in pH during photosynthesis also has a disinfecting effect on the wastewater (de la Noüe *et al*., 1992).

The majority of wastewaters contain very high concentrations of nutrients, particularly total N and total P concentration as well as toxic metals, so there is no requirement for costly chemical-based treatments (Takagiet *et al*., 2006). Sustainable low cost wastewater treatment has been strongly proven by using microalgae (Bashan LE *et al*., 2010). Microalgae grown on wastewater for energy production have been proposed for a long time (Green *et al*., 1996). However, in recent years, microalgae seem to be a favorite candidate for this purpose, due to their ease of cultivation and the favourable possibility of their use as an alternative biomass for bioenergy production. Increase in global warming, depletion of fossil fuel and the need for mitigation of green-house gas (GHS) emissions; make exploration of the feasibility of biological wastewater treatment (I. Rawat *et al*., 2010)

Scandinavian researchers evaluated three wastewater treatment methods based on two frameworks for quantifying the degree to which a process tends toward a sustainable system. The frameworks are known as the "socio-ecological principles" and "energy analysis." Energy analysis locates every resource in an energy hierarchy of the biosphere: "The position of an item in the energy hierarchy is suggested to correspond to the relative influence of that item, on the system of which it is a part." (Groenlund, *et al*. 2004)

Phytoremediation:

Phytoremediation has emerged as the most desirable technology which uses plants for removal of environmental pollutants or detoxification to make them harmless (Cunningham *et al*., 1993). Many living organisms can accumulate certain toxicants to

body concentrations much higher than present in their environments (Kord *et al*., 2010). Thus, the use of plants for the decontamination of heavy metals has attracted growing attention because of several problems associated with pollutant removal using conventional methods. Bioremediation strategies have been proposed as an attractive alternative owing to their low cost and high efficiency (Mejare *et al*., 2001). The algae have many features that make them ideal candidates for the selective removal and concentration of heavy metals, which include high tolerance to heavy metals, ability to grow both autotrophically and heterotrophically, large surface area/volume ratios, phototaxy, phytochelatin expression and potential for genetic manipulation (Cai *et al*., 1995).

Macroalgae have been used extensively to measure heavy metal pollution and marine environments throughout the world. In recent years, several species of the green algae *Enteromorpha* and/or *Cladophora* have been utilized to measure heavy metal levels in many parts of the world (Al Homaidan A *et al*., 2011). The release of free oxygen is of major significance in organically enriched wastewater, promoting aerobic degradation processes by and other microorganisms. Secondly the role of microalgae is the accumulation and conversion of wastewater nutrients to biomass and lipids.

Biofertilizer:

Seaweed has been used as a fertilizer worldwide in coastal regions, mainlyfor its mineral content and to increase the water-binding capacity of the soil. Microalgalspecies that fix nitrogen are important, especially in rice cultivation. Both macro- and microalgae can contain compounds that promote germination, leaf or stem growth, flowering or can be used as a biological protection agent against plant diseases (Pulzand Gross 2004).After the extraction of oil or carbohydrates from both seaweed and microalgae, most of the nutrients are still present in the left-over biomass. Whether left over biomass is used as fertilizer or algaculture nutrient source, anaerobic digestion is a valuable option.

Microalgae are employed in agriculture as biofertilizers and soil conditioners. The majority of cyanobacteria are capable of fixing atmospheric nitrogen and are effectively used as biofertilizers. Cyanobacteria play an important role in maintenance and build-up of soil fertility, consequently increasing rice growth and yield as a natural biofertilizer (Song

et al., 2005). The agricultural importance of cyanobacteria in rice cultivation is directly related with their ability to fix nitrogen and other positive effects for plants and soil. After water, nitrogen is the second limiting factor for plant growth in many fields and deficiency of this element is met by fertilizers (Malik *et al*., 2001).

The use of Blue green algae (BGA), apart from increase in yield and saving of fertilizer nitrogen, the soil physico-chemical properties also improved.There was gradual build up of residual soil nitrogen and carbon,improvement in soil pH and electrical conductivity. The grain quality in terms of protein content improved. Blue green algae belonging to genera *Nostoc, Anabaena, Tolypothrix* and *Aulosira* fix atmospheric nitrogen and are used as inoculants for paddy crop grown both under upland and low land conditions. *Anabaena* in association with water fern *Azolla* contributes nitrogen up to 60 kg/ha/season and also enriches soils with organic matter. A variety of free-living cyanobacteria are now identified as efficient components of cyanobacterial biofertilizers. In addition to contributing nitrogen, cyanobacteria also benefit crop plants by producing various growth-promoting substances.

Aquaculture:

Microalgae feeds are currently used mainly for the culture of larvae and juvenile shelland finfish, as well as for raising the zooplankton required for feeding of juvenile animals (Benemann, 1992, Chen, 2003). They are required for larval nutrition during a brief period, either for direct consumption in the case of molluscs and peneid shrimp or indirectly as food for the live prey, mainly rotifers, copepods and *Artemia*nauplii, which in turn are used for crustaceans and fish larvae feeding (Brown *et al*., 1997, Duerr*et al*., 1998, Muller-Feuga, 2000, Xu *et al*., 2007). The most frequently used species in aquaculture are *Chlorella*, *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema*and *Thalassiosira*(Yamaguchi, 1997, Borowitzka, 1997, Apt and Behrens, 1999, Muller-Feuga, 2000). Microalgae contain essential nutrients which determine the quality, survival, growth and resistance to disease of cultured species.

The importance of the control of microalgal biochemical composition for the success of aquaculture feed chains, opening new perspectives for the study of fish larval nutrition and the development of microalgae based feeds for aquaculture (Fábregas*et al*.,

2001). Aquatic species, such as salmonids (salmon and trout), shrimp, lobster, seabream, goldfish and koi carp under intensive rearing conditions need a supplementation of carotenoids pigments in their diet, to attain their characteristic muscle colour. In addition to pigmenting effects, carotenoids, namely astaxanthin and canthaxanthin, exert benefits on animal health and welfare, promote larval development and provide growth and performance stimulatory effects in farmed fish and shrimp (Baker and Gunther, 2004). *Chlorella vulgaris* biomass proved to be efficient, comparable with synthetic astaxanthinand canthaxanthin, for pigmentation purposes, in rainbow trout (Gouveia*et al*., 1996c, 1997,1998), gilthead seabream (Gouveia*et al*., 2002), ornamental goldfish and koi carp (Gouveia*et al*. 2003, 2005) and shrimps (Passos*et al*., in preparation).

COMMERCIAL APPLICATION

Biodiesel Production:

Biodiesel is a biofuel commonly consisting of methyl esters that are derived from organic oils, plants or animal. The idea of producing biodiesel from microalgae that accumulate high amounts of oil (Sheehan et al. 1998). Many species of algae accumulate large amounts of oils that to a large extent are made up of triacylglycerols consisting of three fatty acids bound to glycerol. The fatty acids are saturated or unsaturated carbon chains of different lengths. Non- or mono unsaturated fatty acids of 16 or 18 carbon length are preferable sources to use for the production of biodiesel. The algal oil is converted into biodiesel through a trans-esterification process. Oil extracted from the algae is mixed with alcohol and an acid or a base to produce the fatty acid methyl esters that makes up the biodiesel (Chisti 2007). A major current problem for the commercial viability of biodiesel production from micro-algae is the low selling price of biodiesel (less than €1 kg-1).

Algae offer many potential advantages such as algae can potentially produce 1 000-4 000 gallon/ acre/yr significantly higher than soybeans and other oil crops. They do not compete with traditional agriculture because they are not traditional foods and feeds and they can be cultivated in large open ponds or in closed photo bioreactors located on non-arable land. They can grow in a wide variety of climate and water conditions; they can utilize and sequester CO2 from many sources. Finally, they can be processed into a broad

spectrum of products including biodiesel via trans-esterification, green diesel and gasoline replacements via direct catalytic hydrothermal conversion, and catalytic upgrading, and bioethanol via fermentation, methane via anaerobic digestion, heat via combustion, bio-oil and bio-char via thermochemical conversion, and high protein animal feed.

There are several ways to convert microalgal biomass to energy sources, which can be classified into biochemical conversion, chemical reaction, direct combustion, and thermochemical conversion. Thus, microalgae can provide feedstock for renewable liquid fuels such as biodiesel and bioethanol.

Thermochemical conversion is a process through which biomass in the absence of oxygen and at high temperature can be converted into various fuels including char, oil and gas. The resulting bio oils present an alternative to liquid biofuels with similarities to petroleum oil (Kishimoto et al. 1994). The process can be subdivided into pyrolysis and thermochemical liquefaction (Demirbas 2000). The former is executed at high temperature (350-530°C) at which a liquid, a gaseous and a solid fraction is produced. The liquid fraction contains an aqueous and a non-aqueous phase (bio-oil or tar), which is recovered. This process requires drying of the biomass while in the latter wet biomass is treated at lower temperature and high pressure (about 300°C and 10 MPa). Since thermochemical liquefaction does not require a drying process but can use the wet material such as algal biomass (water content more than 60%) this technique has a clear advantage compared with pyrolysis. The bio-oil from the processing is composed of all organic compounds in the algae including lipids as well as proteins, fibers, and carbohydrates and therefore gives a higher yield than compared with the content of accumulated lipids in the algae cells. The feasibility of producing liquid fuel or bio-oil via pyrolysis or thermochemical liquefaction of micro-algae has been demonstrated for a range of micro-algae (Dote et al. 1994; Sawayama et al. 1999; Peng et al. 2000; Peng et al. 2001a; Peng et al. 2001b; Tsukahara andSawayama 2005; Demirbas 2006).

Miao *et al.,* (2004) proposed using micro-algae harvested from lakes both to producebio-oil via fast pyrolysis and as an environmental solution to reduce algae blooms. Up to 24% of the dry biomass was recovered as bio-oil. The pyrolysis oils had better properties than the oil from lignocellulosics, however, they still have a much higher oxygen

content compared to fossil oil and their heating value is low with 29 MJ kg-1 compared to 42 MJ kg-1 of fossil oil.

Food Colorants:

Microalgal pigment has commercial uses as a natural food coloring and cosmetic ingredient. Some microalgae contain substantial amounts of Carotene (besides beta carotene). Other types of coloring appear in microalgae as well. Beta carotene is used as a food coloring (with a major application in providing the yellow color to margarine), as a food additive to enhance the color of the flesh of fish and the yolk of eggs, and to improve the health and fertility of grain-fed cattle (Borowitzka and Borowitzka, 1987).

Natural Beta Carotene has physical properties that make it superior to synthetic. In particular, natural Beta Carotene is fat soluble. Beta Carotene is effective in controlling cholesterol and in reducing risks of heart disease. These new findings make Beta Carotene much more valuable and are likely to increase the demand for the product. By being fat soluble, the natural Beta Carotene is a much superior anticarcinogen and an antiheart disease agent. Thus, the new findings of these desirable medical properties are likely to increase even more the demand and desirability of natural Beta Carotene. The potential of micro-algae as a source of food coloring is limited, however, because algal derived food coloring is not photostable and the color tends to bleach with cooking. Nevertheless, in spite of this limitation, the potential market for micro-algae-derived food coloring is vast. *Dunaliellasalina*is grown for a source of the photosynthetic pigment, beta-carotene. Beta-carotene is used as an orange dye and as a vitamin C supplement (Indira Priyadarshani and BiswajitRath, 2012).

Nutrient Supplement:

Algae are cultivated and used in nutrition worldwide. They are an important source of vitamins, minerals, proteins, polyunsaturated fatty acids, antioxidants, etc (Pulz and Gross, 2004). Microalgae biomass represents a valuable source of nearly all essential vitamins (*e.g.* A,B1, B2, B6, B12, C, E, nicotinate, biotin folic acid and pantothenic acid) and a balanced mineral content (*e.g.* Na, K, Ca, Mg, Fe, Zn and trace minerals) (Becker, 2004). The high levels of vitamin B12 and Iron in some microalgae, like *Spirulina*, makes

them them particularlysuitable as nutritional supplements for vegetarian individuals. The vitamin content of an algadepends on the genotype, the stage in the growth cycle, the nutritional status of the alga, the light intensity (photosynthetic rate). The vitamin content is therefore amenable to manipulation by varying the culture conditions as well as by strain selection or genetic engineering. However, vitamins cell content fluctuates with environmental factors, the harvesting treatment and the biomass drying methods (Brown *et al*., 1999, Borowitzka, 1988).

Mineral rich seaweed has been incorporated in commercial salmon feeds at 15 % in lieu of manufactured vitamin and mineral pre-mixes (Kraan, Mair 2010). Final tests suggested that salmon fed the "seaweed" feeds appeared to be healthier, more active; flavour and texture were improved which may have been due to the bromophenolic compounds found in seaweeds. Elsewhere, *Enteromorphaprolifera*and *Cladophora sp*., when added to the feeds of laying hens, positively influenced egg weight and egg shell thickness (Michalak et al. 2010).

The vitamin content of algal biomass can vary significantly among species. Ascorbic acid shows the greatest variability according to Brown and Miller (1992), although this may have been due to differences in processing, drying and storage of algae, as ascorbic acid is very sensitive to heat. This highlights the drawback of supplying essential micronutrients via natural sources, i.e. there is too much variability arising from the combined effects of different algal species, growing season, culture conditions, and processing methods to reliably supply the required micronutrients in a pre-determined fashion. Accordingly, algal biomass mainly offers a supplementary source rather than a complete replacement for manufactured minerals or vitamins in animal feeds.

The high protein content of various microalgae species is one of the main reasons toconsider them as an unconventional source of protein (Soletto*et al*., 2005), well-illustrated by the great interest in microalgae as single cell protein (SCP) during the 1950s. In addition, the amino acid pattern of almost all algae compares favorably with that of other food proteins. Since the cells are capable of synthesize all amino acids, they can provide the essential ones to humans and animals (Guil-Guerrero *et al*., 2004). As other bioactive compounds synthesized by microalgae, amino acids composition, especially the free amino acids, varies greatly between species as well as with growth conditions and growth phase

(Borowitzka, 1988). Protein or amino acids may therefore be by-products of an algal process for the production of other fine chemicals, or with appropriate genetic enhancement, microalgae could produce desirable amino acids in sufficiently high concentrations (Borowitzka, 1988).

Microalgae

Microalgae are an enormous biological resource, representing one of the most promisingsources for new products and applications (Pulz and Gross, 2004). They can be used to enhance the nutritional value of food and animal feed, due to their well-balanced chemical composition. Moreover, they are cultivated as a source of highly valuable molecules such as polyunsaturated fatty acids, pigments, antioxidants, pharmaceuticals and other biologically active compounds. The application of microalgae biomass and/or metabolites is an interesting and innovative approach for the development of healthier food products. Some of the most biotechnologically relevant microalgae are the green algae (Chlorophycea) *Chlorella vulgaris*, *Haematococcuspluvialis*, *Dunaliellasalina*and the Cyanobacteria *Spirulina maxima* which are widely commercialized and used, mainly as nutritional supplements for humans and as animal feed additives. *Spirulina platensis*, a blue-green alga is gaining worldwide popularity as a food supplement, being one of the most nutritious food known to man. It is gaining worldwide popularity as a food supplement. It has been shown to be an excellent source of proteins (Colla*et al*., 2007), polyunsaturated fatty acids (Sajilata, 2008), pigments (Rangel-Yagui*et al*., 2004; Madhyastha and Vatsala, 2007), vitamins and phenolics (Colla*et al*., 2007; Ogbonda*et al*., 2007). Today the major use of *Spirulina* is for the extraction of phycocyanin, a blue photosynthetic pigment. Another potential microalgae used as food is the green algae *Chlorella*. Now a days *Chlorella*, like *Spirulina* is mainly sold in health food stores and as a fish food.

The major economic important product of *Chlorella* are several by-products that are used in fruit and vegetable preservatives (Hills and Nakamura, 1976). There is another most important microalgae under modern cultivation is*Dunaliellasalina*. This species is grown for a source of the photosynthetic pigment and beta-carotene. Beta-carotene is used as an orange dye and as a vitamin C supplement. At present microalgal market is

dominated by *Chlorella* and *Spirulina* (Becker, 2004; Pulz and Gross, 2004), mainly because of their high protein content, nutritive value, and moreover they are easy to grow. The biomass of these algae is marketed as tablets, capsules and liquids which are used as nutritional supplement. Microalgae are also added to pasta, snack foods or drinks either as nutritional supplements or natural food colourants (Becker, 2004). Functional food oil, rich in fatty acids and antioxidants, coloured with pigments (carotenoids) extracted with supercritical CO_2 from the microalga *Chlorella* vulgaris, was produced, having in view its use in food industry especially for derived seafood. Microalgal biomass contains three main components: proteins, carbohydrates, and lipids (oil) (Um and Kim, 2009).

Macroalgae:

Dietary seaweeds provide all essential minerals. No land plant even remotely approaches seaweeds as sources of metabolically required minerals (Bergner, 1997). Seaweeds can provide minerals often absent from freshwater and food crops grown on mineral-depleted soils. Seaweeds are 20-50% dry weight mineral (Kazutosi, 2002). The mineral macronutrients include sodium, calcium, magnesium, potassium, chlorine, sulphur and phosphorus; the micronutrients include iodine, iron, zinc, copper, selenium, molybdenum, fluoride, manganese, boron, nickel and cobalt. Seaweed has such a large proportion of iodine compared to dietary minimum requirements, that it is primarily known as a source of this nutrient (SubhutiDharamananda. 2010).

Seaweed contains several vitamins. Red and brown algae are rich in carotenes (provitamin A) and are used, in fact, as a source of natural mixed carotenes for dietary supplements. The content ranges from 20-170ppm. The vitamin C in red and brown algae is also notable, with contents ranging from 500-3000ppm. Other vitamins are also present, including B12, which is not found in most land plants. Seaweed has very little fat, ranging from 1-5% of dry matter, although seaweed lipids have a higher proportion of essential fatty acids than land plants. The soluble fiber fraction accounts for 51-56% of total fibres in green (ulvans) and red algae, carrageenans and xylans) and for 67-87% in brown algae (laminaria, fucus, and others). Soluble fibres are generally associated with having cholesterol-lowering and hypoglycemic effects (SubhutiDharmananda, 2010).

Macro-algae have long been used for the production of phycocolloids such asalginates, carrageenans or agars (Lewis et al. 1988 Radmer 1996;Renn 1997). These polymers are either located in cell walls or within the cells serving as storage materials. A characteristic of marine algae is the abundance of sulphated polysaccharides in their cell walls. The group of phycocolloid polymers commonly termed hydrocolloids includes thealginates, carrageenans and agars. They make up the major industrial products derived from algae (Radmer 1996, Pulz and Gross 2004). The raw materials for the production of hydrocolloids are macro-algae (red and brown seaweeds). Hydrocolloids are polysaccharides that are not found in terrestrial plants, although polymers with similar properties can be produced by certain land plants. The polymers are used in many food and industrial products to thicken, emulsify and stabilize. Hydrocolloids can be dissolved in warm water and will form a gel on cooling. The gel properties can be modified by varying the concentrations of metal ions present, thetemperature and the pH, making them suitable for various applications.

Alginates are polymers from the cell walls of a wide variety of species of the brownalgae, particularly species of *Laminaria*, *Macrocystis* and *Ascophyllum*. They are polymers of composed of D-mannuronic acid and L-guluronic acid monomers. The alginates are extracted from the cell walls using hot alkali (sodium carbonate) (Radmer 1996). Alginates are commonly used in the food and pharmaceutical industries as stabilisers for emulsions and suspensions, e.g. ice cream, jam, cream, custard, creams, lotions, tooth paste, as coating for pills. They are also used in the production of paint, construction material, glue and paper, the oil, photo and textile industry (Radmer 1996). Brown seaweeds for alginate production are harvested from the wild and not cultivated for this purpose (McHugh 2003). Although these seaweeds are cultivated to produce food in China, their cultivation to provide raw material for industrial uses would be too expensive (McHugh 2003).

Carrageenans are used in the food, textile and pharmaceutical industry and function as a stabiliser for emulsions and suspensions. carrageenans are especially used in chocolate milk, ice cream, evaporated milk,puddings, jellies, jams, salad dressings, dessert gels, meat products and pet foods, dueto their thickening and suspension properties. Several potential

pharmaceutical uses of carragenans (like antitumor, antiviral, anticoagulant and immunomodulation activities)(Cardozo, Guaratini et al. 2007) have also been explored.

Agar is made from seaweed and is used in a wide range of applications: in foodproducts (such as frozen foods, bakery icings, meringues, dessert gels, candies and fruit juices), industry uses (like paper sizing/coating, adhesives, textile printing/dyeing, castings, impressions), in biological culture media, in molecular biology (more specifically agarose, used for separation methods) and in the medical/pharmaceutical field (to produce bulking agents, laxatives, suppositories, capsules, tablets and anticoagulants) (Cardozo, Guaratini et al. 2007).Like carrageenans, agars are used as stabilisers for emulsions and suspensions and as gelling agents. About 90% of the agar produced was for food applications and the remaining 10% were used forbacteriological and other biotechnological uses.

ALGAE IN PAPER PULP

Fibres for paper

Most plant cell walls consist of cellulose, but in algae cell coverings are very diverse.Some algae species have intracellular walls, or scaly cell walls made of deposits of calcium carbonate or silica, but most algae derive structural strength from continuoussulphated polysaccharides in marine algae; other possibilities being cellulose, carrageenan, alginate and chitin (Okuda 2002).

Cellulose-containing algae can potentially be used as a renewable feedstock for paperproduction as the strong green colour of algae is more difficult to bleach than wood fibres but, although algae are generally known for their low cellulose and hemicellulose content, there are a few examples of research into the use of algae as a non-wood fibre source. Ververis*et al.* (2007) used a mix of algae taken from a municipal waste water treatment as 10% of the pulp mix, resulting in a significant increase in the mechanicalpaper strength and a decrease in paper brightness. The best result of pure algae-paper approached standard paper quality, showing lower bursting, tearing and folding strengths. Mixing with softwood pulp improved the paper to Kraft quality (Chao, Su et al. 1999; Chao, Su et al. 2000; 2005). This algae is filamentous (forms long threads) and is therefore much easier and cheaper to harvest than unicellular algae. Another benefit is the salt tolerance of

Rhizoclonium, ranging from 1.0 to 3.3 % salt, with an optimum at 2.0% salt (seawater averages 3.4% salt). At this optimum, most naturally occurring freshwater algae will not be able to grow. *Chaetomorphalinum* and *C. Melagonium* have similar cellulose contents (Chao, Su et al. 1999), while *Vaucheria* species can contain about 90% cellulose in their cell wall (Parker, Fogg et al. 1963) in (Okuda 2002).

Biologically different from algae and seaweed, but similar in cultivation and processingare certain aquatic plants. These may also have high productivities and may be grown on waste streams, and since they are closer to land plants, have high fibre contents. Joedodibroto (1983) has investigated several aquatic plants and concluded that all three weeds produced moderate quality paper pulp. Water hyacinth gave good folding and tearing resistance, but the processing of material from this plant was rather difficult. Other investigators reported that paper from 75% water hyacinth pulp and 25% bamboo pulp gave a high strength and also good greaseproof properties to the paper (Goswami and Saikia1994).

There is some promising research on the utilisation of aquatic biomass for paper pulp, a development that deserves future attention, from economical, renewable and quality points of view. However this concept has not moved beyond the research stage yet and it is unclear when it will be commercialised.

World paper consumption was about 300 million tons in 1996/97 and is expected to rise above 400 million tons by the year 2010 (Hurter and Riccio, 1998). In view of the shortage of conventional raw materials for pulping and the increasing demand for paper products, new raw materials for pulp production such as non-wood fibers are being investigated worldwide (Ververis*et al*., 2004). Some of the new materials are either filamentous algae that can be used as the main raw material for papermaking (Kiran*et al*., 1980; Sakai *et al*., 1996; Chao *et al*., 2000) or algal biomass used as a supplement in softwood or hardwood pulps (Nicolucci*et al*., 1994).

The major problems in producing paper from filamentous algae are (i) the isolation of certain species and growth of biomass in culture solutions inevitably increase the process cost and (ii) the relatively poor mechanical strength of the algal pulps (Chao *et al*., 2000). On the other hand, mixing algal biomass with conventional paper pulp seems a more promising method for exploiting algae from eutrophic waters (Nicolucci*et al*., 1994).

The use of Red algae for producing raw materials for papermaking with regard to their physical components which contains large amounts of mucilaginous materials such as agar or carrageenan, which can be easily extracted with hot water, and small amounts of solid materials, which are endofibers. After the extraction of the mucilaginous materials, the remaining material mostly consists of endofibers (also known as rhizoidal filaments, rhizine, internal filaments, and hypha (Lee *et al*., 2003), which are then bleached to make bleached red algae pulp. These endofibers could be used as a new type of raw material for papermaking, which would be more abundant worldwide than wood fibers. The average growing rate of some red algae in the sea is around 3–10% per day (dry weight) during the growing season (Gel-Or *et al*., 2004; Ohno*et al*., 1996; Felicini*et al*., 1994), and red algae grow under the sea surface worldwide except in the arctic areas. There is no quantitative limit on the supply of endofibers or red algae pulp as long as investment for their cultivation in the sea and the necessary processing facilities are available.

Alginates in Paper Industry:

Alginates partially complexes with calcium (such as forming a loose gel) and when mixed with starch was proposed to get high water retention in paper coating (Joyce, M. and Gilbert, S.A.) thus finds application in paper industry for supplementing wood pulp.

QUALITY OF PAPER:

Testing the quality of the paper by physical and optical properties: Basis weight, bulk, brusting strength, tearing strength, tensil strength, breaking length, smoothness, porosity, brightness.

Basis weight:

It is the “weight of paper per unit area”. It is expressed as grams / sq. meter.It influences all paper properties. According to end use, gsm requirement varies.

Bulk:

It is the volume of paper per unit weight and is reciprocal of density. It unit is cc/g. Bulk=Thickness in Microns / Basis wt in gsm**.** This influences all other properties**.** High bulk gives good resistance and superiorprinting qualityBulk decreases with improved

smoothness**.** Low Bulk paper has low opacity**.** To maintain Bulk, proper furnish mix, low refining, low calendering and low ash content are being maintained**.**

Bursting strength:

Bursting strength is defined as the hydrostatic pressure in Kilo-Pascal, or Kilo- Newton per square meter required to produce rupture of the material when the pressure is increased at a controlled constant rate through a rubber diaphragm.To compare at various gsm of paper it is expressed as Burst factor.

Burst factor = (Bursting Strength X 10.2)/gsm

This is an important property for packing grade paper**.** By proper refining the stock and by adding starch this property is being maintained.

Tearing strength:

Tear is the force required to tear a single sheet of paper through a specified distance and is expressed as Tear Factor.

Tear Factor = (Tearing Strength X10.2)/ gsm**.**

This is an important directional property for Tissue, Wrapping, Packing and Specifically for printing paper in reel form.Proper furnish mix and refining ensures required tearing strength.

Tensile strength:

Tensile strength is an indicative of the serviceability of many papers, such as web printing, wrapping, bag and gummed tape and creped papers such as cable wrapping, tissue and toweling, which are subjected to direct tensile stress . The tensile strength tells how well the paper will resist breaking during a process such as printing.Tensile strength measures the force required to break a standard width strip (15 mm).

Breaking Length:

Breaking length which is a strength to weight ratio which indicates the length of a strip of paper required to cause the strip to break under its own weight when hang freely (OR)The breaking length of a paper is the length of a uniform strip that is just sufficient to break under its own weight when suspended at one end. This is the length of a strip of paper expressed in meters, which would break on its own weight when suspended vertically.

Tensile strength of paper is always greater in the machine direction than in the cross direction because of the greater alignment of fibers in the machine direction. The ratio between machine and cross direction tensile strengths is an indication of the squareness of the sheet. By proper refining of stock and optimising the ash content this property is controlled.

Smoothness:

It is measured by the amount of air escaping along the surface of the paper under a specified weight and expressed as millilitre / minute in Bendtsen. Smoothness is concerned with the surface contour or mechanical perfection of the paper surface.An ideally smooth surface is one in which all the surface elements lie in one plane.The smoothness of a real surface such as that of paper can thus be easily defined as the closeness of its surface to the plane surface.

Smoothness means freedom from lumps, wire and felt marks, fuzziness, foreign matters, inter fiber voids, crush, cockles, mechanical damage (scabs, press and calender cuts) and incompressibility and other gross surface perfections.Generally rough papers require more ink in printing causing excessive show through and poor halftone dot formation. Because of more ink carried on the plate, the ink mileage is reduced.

Porosity:

Porosity is the rate of passage of air through the paper under the influence of a difference in pressure. Therefore, porosity indicates the structural characteristics of the paper and in particular, the diameter and spacing of the capillary air passage. Porosity is inversely proportional to density.Porosity is a property of direct importance in writing and printing papers since it is a factor in the absorption of inks.Lesser the porosity of paper, printing ink consumption will be less.

Brightness:

It is the percentage of blue light reflected of a sample measured at an effective wavelength of 457nm and it is distributed throughout the spectral range of 400-500 nanometers.Brightness is also well suitable for measuring the permanence of paper, since

the change in the color of paper on aging or thermal degradation is greatest in the blue and violet regions of the spectrum where brightness is measured.

Papermaking is the formation of a matted or felted sheet, usually made of cellulose fibers, from water suspension on a wire screen. Paper is the basic material which is used for written communication and for dissemination of information. In addition, paper and paperboard also provide materials for hundreds of other uses, such as wrapping, packaging, toweling, insulating, and photography. The word paper is derived from the name of the reedy plant Papyrus, which grows abundantly along the Nile River in Egypt. Papyrus was the most widely used writing material in ancient times, and many Papyruses still survive (Kennath W. Britt, 1965). Papermaking is one of the inventions by Chinese. 105 A.D. is often cited as the year in which the papermaking was invented and the historical records show that the invention of paper was reported to Eastern Han Emperor Ho-di by Ts'ai Lun, an official of the Imperial Court. The first paper was apparently made from true hemp (Atchison and McGovern,1989).

Paper production in India

Paper is one of the core industries and is linked to the basic needs. Paper is pre-requisite for education and literacy and is an index of advancement in these two fields as well as the overall well being of the society. Paper manufacturing has been carried on in India since tenth century as a small cottage industry by the traditional craftsmen called kagzis. They used gunny bags, rags, ropes, etc. for making handmade paper (Alka Subramanian, 1987). The beginning of modern paper industry goes back to 1816 when a factory was set up near Chennai. This venture proved abortive. The first successful effort was made in 1870 with the setting up of the Royal Bengal Paper mills at Ballyganj near Kolkata. Soon after, a number of units were setup (Srivaram Reddy and Mohan Reddy, 1989). At the beginning of 20^{th} century, India's production of paper stood as 19,000 tonnes per year and it was raised to 58.90 lakh tonnes in the year 2007 – 08 (Satyasundaram, 2009).

Pulp and paper manufacturing

Source of fiber, chemicals, energy and water are the four major inputs of papermaking process.

Source of Fiber

Wood is a primary source of fiber and is a composite material consisting of flexible cellulose fibers. The three main components present in wood are cellulose, hemicelluloses and lignin. Although the concentration of these three components can vary from species to species, the proportions are roughly 50% cellulose, 25% hemicelluloses and 25% lignin.

Cellulose

Cellulose is a very linear molecule composed of repeated glucose subunits. Cellulose fibers have a slight negative (anionic) charge. The ionizing (acidic) groups on cellulose originate from cell wall constituents or are introduced during pulping and bleaching. The number of charged groups, primarily carboxyl groups, varies between 2 and 30 meq/100 g pulp for different types of papermaking fibers. Glucuronic acid residues on xylan remaining in the pulp and carboxyl groups in residual lignin are an additional source of acidic groups in alkaline de-lignified (Kraft) pulp. The charged groups on fibers affect their swelling properties and electrochemical interactions with chemical additives, including starch (Hans W. Maurer, 2009).

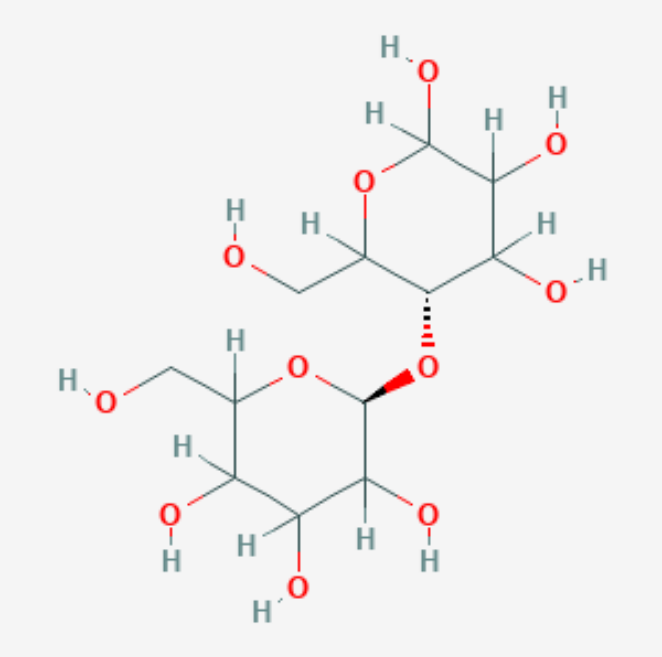


Figure 1: Structure of cellulose

Cellulose - Source: www.pubchem.ncbi.nlm.nih.gov

Hemicelluloses

In the production of cellulose-based materials, to modify the interactions between the fibers or to adjust its compatibility with other materials, cellulose fibers usually have to be chemically functionalized. An attractive path is the use of hemicelluloses, e.g., xylans which have a considerable potential in the development of new high-performance materials with low environmental impact. The interaction between hemicelluloses and cellulose is possible because the linear xylan backbone allows a partial alignment and formation of hydrogen bonds to cellulose microfibrils. The xylans retained on the cellulose surfaces formed nano and micro-sized particles by surface modification of cellulose fibers. This is of practical importance that xylan-modified lingo cellulosic fibers show improved wetting and liquid-spreading properties. Thus, the study of xylan adsorption on cellulose may be a method of preparing new materials and composites (Jun-Li Ren, Run-Cang Sun, 2010).

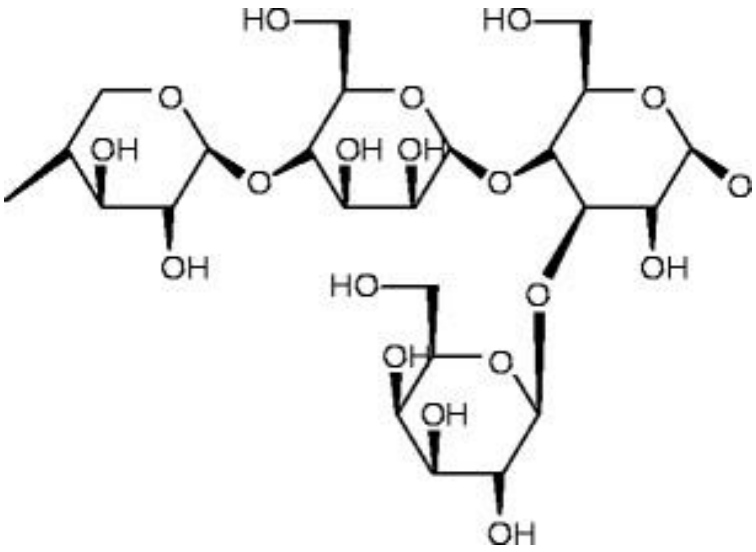


Figure 2: Structure of Hemicellulose

Source: www.researchgate.net

Lignin

Lignin is a complex of phenyl propanoid polymer that surrounds and gives strength to the cellulose-hemi cellulose framework. Lignin is composed of three phenol-based building blocks that polymerize in a completely random fashion which is very difficult to degrade. When slow degradation takes place phenolic compounds which are generally toxic, are released. The aromatic content of lignin is approximately 51% which is expressed as monomeric phenol. It is the phenolic compounds released from lignin during the chlorine bleaching of pulp that are responsible for a large percentage of the toxic compounds released in pulp mill effluents.

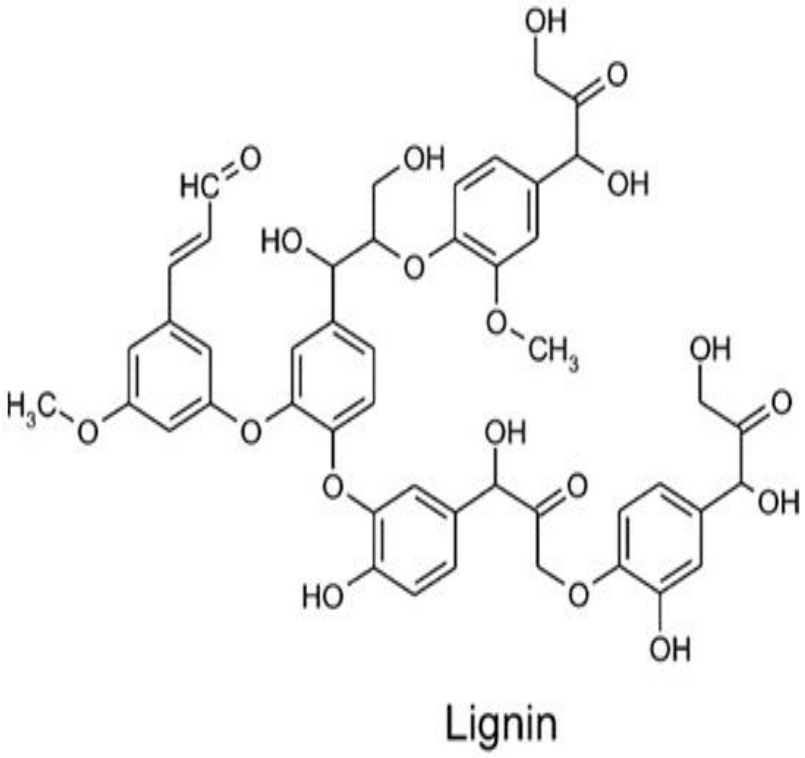


Figure 3: Structure of lignin

Source: www.free-stock-illustration.com

Chemicals

Wood is a complex mixture composed primarily of a polymer called cellulose. The cellulose fibers in wood are bound together by another polymer called lignin. Paper makers must remove the lignin from the wood pulp. The most common approach is the Kraft process, in which wood chips are combined with a mixture of sodium hydroxide and sodium sulfide in water at high temperature and pressure. Under these highly basic conditions, the negatively charged sulfide ions react with the lignin polymer chains to break them down into smaller subunits so the cellulose fibers are freed up for further use. One such alternative is acid sulfite pulping, where a mixture of sulfurous acid and either sodium, magnesium, calcium or ammonium bi-sulfite in water dissolves the lignin to free up the cellulose fiber. Yet another alternative is neutral sulfite semi chemical pulping, where the chips are mixed with a mixture of sodium sulfite and sodium carbonate in water and cooked. Unlike the others, this process only removes a portion of the lignin, so after pulping the chips must be shredded mechanically to remove some of the remaining polymer (John Brennan, 2017)**.**

Energy

Electricity is used throughout the typical pulp and paper mill to power motors and machine drives, conveyors, and pumps, as well as building operations such as lighting and ventilation systems. The largest use of fuels is in boilers to generate steam for use in pulping, evaporation, papermaking, and other operations. Black liquor is the dominant fuel for boilers in the pulp and paper industry, followed by hog fuel and natural gas, and to a lesser extent, coal (Klass Jan Kramer *et al*., 2009).

Water

Water is used in significant quantities in all major process stages of pulp and paper manufacturing, from raw materials preparation (e.g., wood chip washing) to pulp washing and screening to the paper machine (e.g., fabric showers). Large amounts of water are also used to generate steam for use in processes and on-site power generation, for process cooling, for materials transport, for equipment cleaning, and for general facilities operations. Water is therefore a resource that is as critical as energy in the pulp and paper

making process, and one that accounts for considerable operating costs (Bryant *et al*., 1996).

Manufacturing process

The pulp and manufacturing process can be divided into four steps: wood handling and debarking, pulping, bleaching and paper making.

Wood handling and debarking

Pulp manufacturing starts with raw material preparation, which includes debarking (when wood is used as raw materials), chipping, chip screening, chip handling and storage, and other processes such as depithing (e.g., when bagasse is used as the raw material) (Biermann, 1996a; Gerald, 2006; Gullichsen, 2000). Log debarking is necessary to ensure that the pulp is free of bark and dirt. Both mechanical and hydraulic bark removal methods are in common use.

The barking drum is the most common form of mechanical debarking. Bark is removed from the logs by friction created from the rotating drum action as the logs rub against each other. In wet drum barkers, water is added to the early solid steel portion of the drum to help loosen the bark. The remaining portion of the drum has slots to permit the removed bark to fall out while the log continues on through. In dry drum barkers, the entire length of the drum has slots for bark removal.

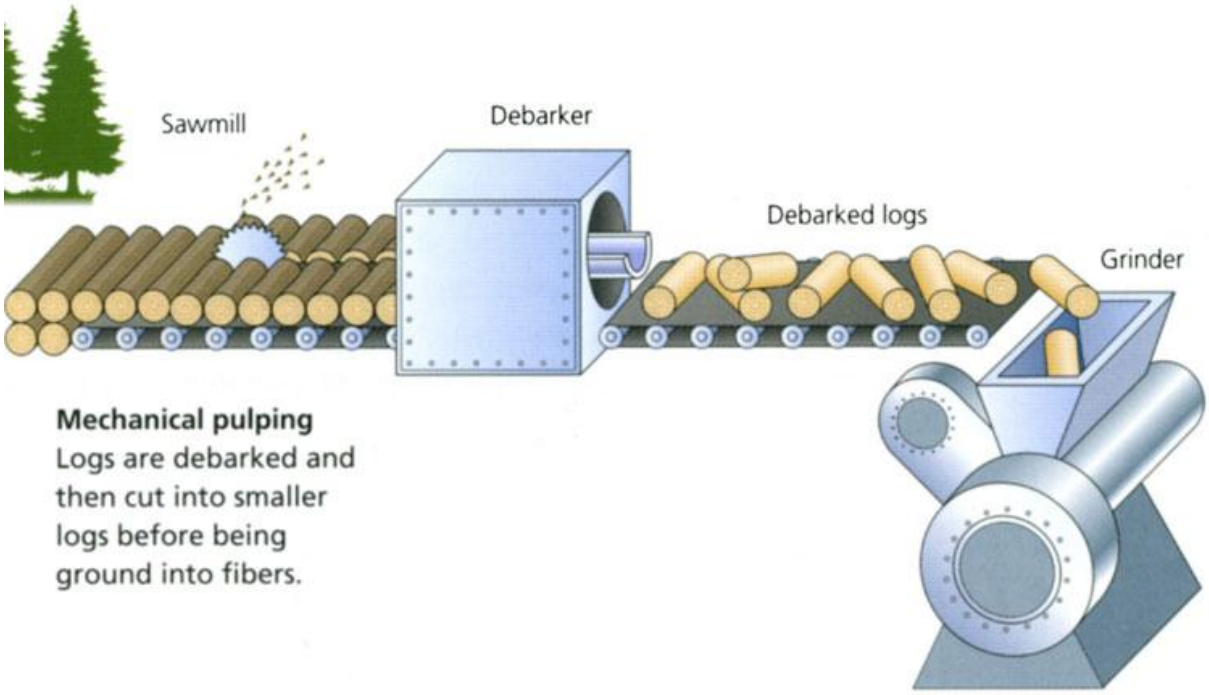


Figure 4: Barking of wood

Source: www.csun.edu.jpg

Dry drum barkers are longer in length and rotate much faster than wet-type drum barkers. The bark from dry drum barking can be fired directly into bark-burning furnaces, while bark from a wet system must be collected in a water flume, dewatered and pressed before burning. Drum barkers usually create about 4–5% wood waste and cause broomed ends on the logs that produce inferior wood chips for pulping. They are relatively low-cost devices but have high power consumption (Russel, 2006).

Pulp making process

Manufacturing of pulp starts with raw material preparation (Smook 1992a; Biermann 1996a). Cellulosic pulp is manufactured from the raw materials, using chemical and mechanical means. The manufacture of pulp for paper and cardboard employs mechanical (including thermo mechanical), chemi-mechanical, and chemical methods.

Chemical pulping

Chemical pulp is produced by combining wood chips and chemicals in large vessels known as digesters where heat and the chemicals break down the lignin, which binds the cellulose fibers together, without seriously degrading the cellulose fibers. Chemical pulp is used for materials that need to be stronger or combined with mechanical pulps to give a product of different characteristics. The kraft process which uses sodium hydroxide(NaoH) and sodium sulphide to pulp wood which is the dominant pulping process in paper industry(Honghi Tran, 2004). The pulping digester operates at about 175 °C for 2–5 h. Kraft pulping has replaced the older soda process, in which sodium hydroxide was the primary pulping chemical. The addition of sodium sulfide in kraft pulping promotes ether cleavage and inhibits undesirable condensation reactions. Sulfite pulping is an acidic process that produces lower yields than the kraft process, and the fibers are also weaker. Its advantage is that a greater percentage of lignin is removed, making the resulting fibers more suitable for high-quality paper and ‘chemical cellulose (M. T. Holtzapple, 2003).

Mechanical pulping

Mechanical pulping separates fibers from each other by mechanical energy applied to the wood matrix causing the gradual break of the bonds between the fibers and the release of fiber bundles, single fibers, and fiber fragments (Smook 1992b ; Biermann 1996b). It is the mixture of fibers and fiber fragments that gives mechanical pulp its favorable printing properties. In the mechanical pulping, the objective is to maintain the main part of the lignin in order to achieve high yield with acceptable strength properties and brightness.

Mechanical pulps have a low resistance to aging which results in a tendency to discolor. The main processes are Stone Ground wood Pulping (SGW), Pressure Ground wood Pulping (PGW), Thermo-Mechanical Pulping (TMP), or Chemi-Thermo-Mechanical Pulping (CTMP). The ground wood pulping process grinds wood into pulp. Usually this involves taking a log and pressing it against a rotating surface to grind off small pieces. The ground wood pulp is then often cooked to soften it. This pulp is used in newsprint and other low cost book grades where it contributes bulk, opacity, and compressibility. Ground wood

pulp is economical since all the wood is used; however, it contains impurities that can cause discoloration and weakening of the paper (Hultman, 1997).

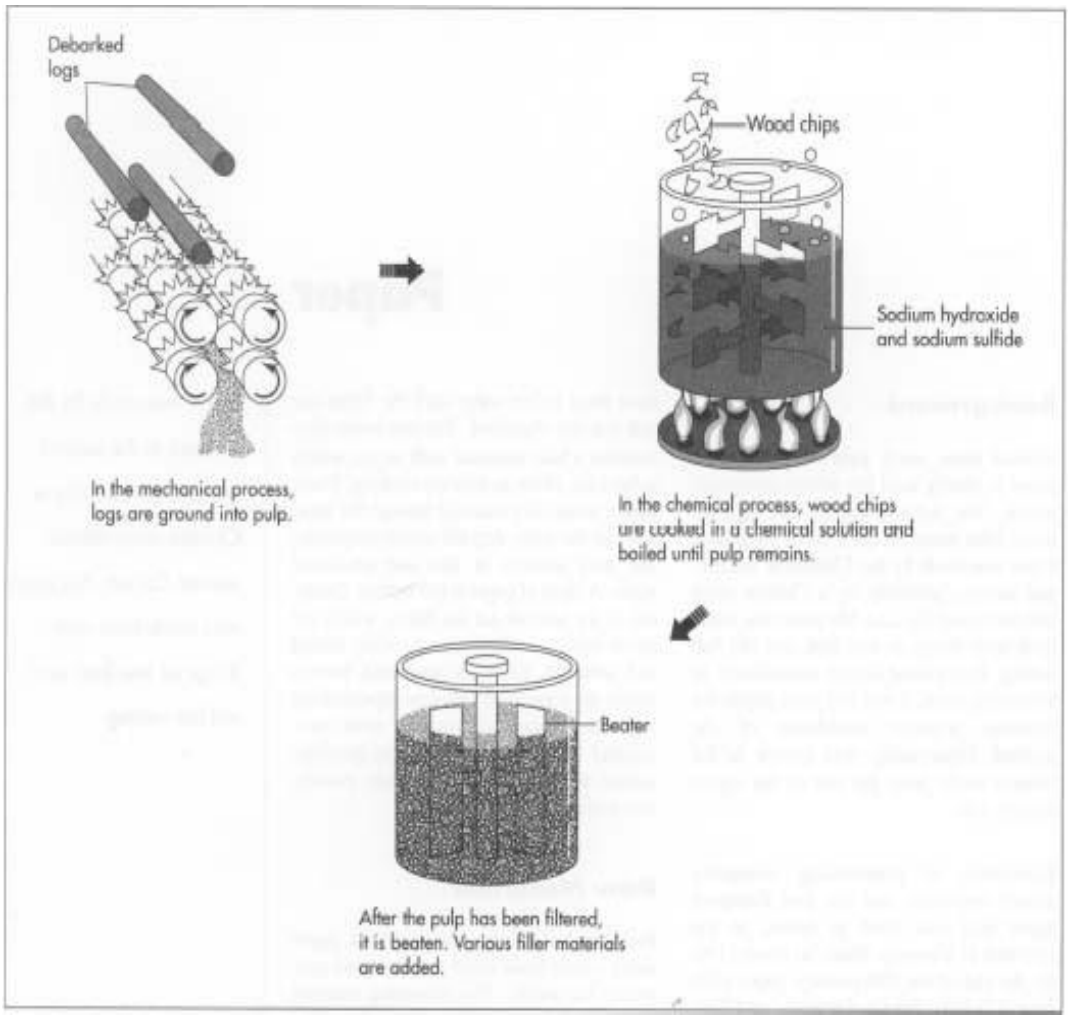


Figure 5: Mechanical pulping

Source: www.madehow.com

Chemi-Mechanical pulping

The chemi-mechanical pulping comprises four main operations, viz. chipping, grinding, leaching, and beating. For chemi-mechanical pulps, the influence of caustic soda charge applied in the leaching process upon the bending stiffness and bending modulus of elasticity in the region of reverssible deformation was investigated by using a three-point

loading method. The preliminary results revealed that the bending modulus of elasticity increases with increasing caustic soda charge. Similarly, the bending stiffness of hand sheets having lower basis weight slightly increases with increasing alkaline charge, whereas, for higher basis weight, the effect of alkaline charge on the bending stiffness is ambiguous. Of course, the tensile strength of chemi-mechanical pulps was much lower than that published for kraft pulp or waste paper The results obtained for rapeseed straw were compared with those measured for unbleached spruce groundwood and for moulded fiber products, which were published earlier. The bending stiffness and bending modulus of elasticity were much greater than those for moulded fiber products made from waste paper. Besides the strength characteristics, the degree of polymerization and bonding properties of rapeseed straw pulp was investigated (Potucek and Gurung, 2014).

Bleaching

Bleaching is the treatment of cellulosic fiber with chemicals to increase brightness. Brightness may be achieved by either lignin removal (delignification) or lignin decolonization. Lignin remains a major constituent of pulp even after digestion by chemical pulping (Douglas W. Reeve, 1987).

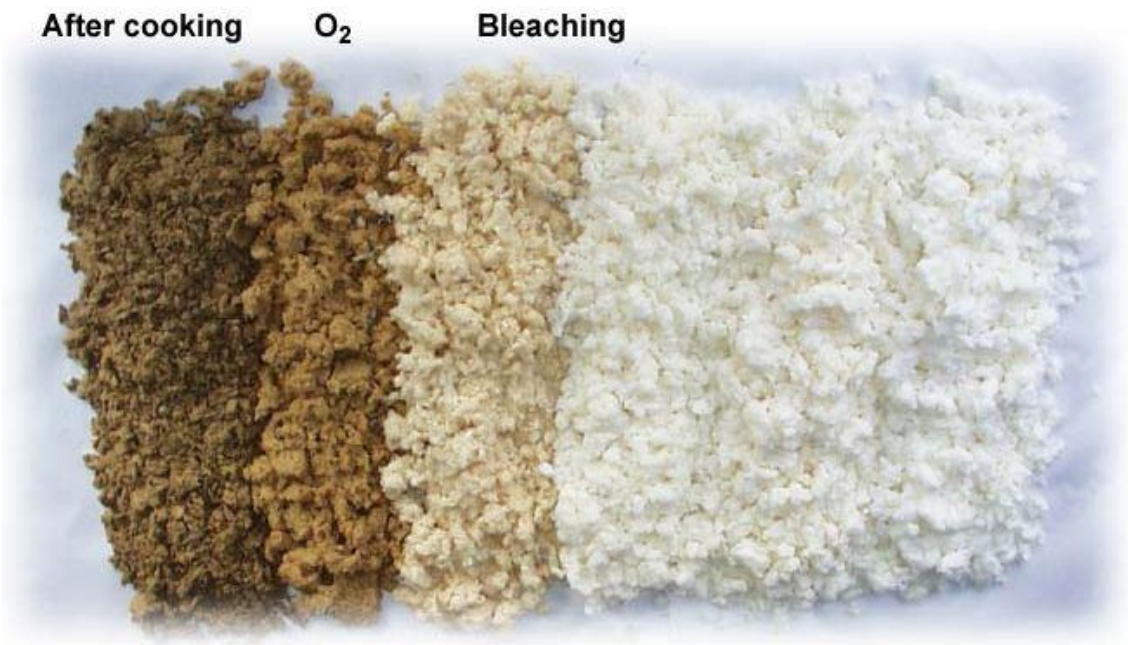


Figure 6: Bleaching process

Source: www.cheya.blogspot.in

In kraft pulping, about 90% of wood lignin is solubilized to the cooking liquid. The remaining 10% of lignin is mainly responsible for the brown color of the kraft pulp and unbleached paper. The primary goal of bleaching is to remove the residual lignin from the pulp as selectively as possible, without degrading the pulp carbohydrates, particularly cellulose, which would decrease the viscosity and strength. Until 1990s, chemical pulp bleaching was carried out with elemental chlorine and chlorine dioxide. Tightened environmental regulations have driven the pulp and paper industry to implement alternative bleaching processes with decreased formation of chlorinated organic compounds.

Today, elemental chlorine has been replaced in bleaching with other chemicals, such as chlorine dioxide, ozone, oxygen, peroxide, or peroxyacids. Alternative bleaching sequences which used are the oxygen chemical-based totally chlorine-free (TCF) and particularly the chlorine dioxide-based elemental chlorine-free (ECF) sequences, are increasingly used in bleaching process. Oxygen delignification is mainly used as a pre bleaching stage before the ECF and TCF bleaching sequences (L.Viikari, A. Ragauskas, 2009).

Paper making

Once the fibers have been sufficiently dewatered that they begin to bond to form paper, they move on to the press section of the paper machine. Here the paper is pressed to remove water, which promotes further bonding between fibers. As it moves through the press section, the paper is supported by rolls and press fabrics which absorb water from the sheet at the press nips. The bonded and dewatered sheet then proceeds to the so-called dry end of the paper machine for further drying and finishing operations. The press section has historically been the target of many energy efficiency improvements in papermaking, because the drier the paper is leaving the press section, the less energy it consumes in the drying section (Klaas Jan Kramer, *et al*., 2009).

Drying process

Dry end processes include drying, calendering, and reeling. In the drying section, steam heated rollers compress and further dry the sheet through evaporation, which facilitates additional bonding of fibers. The drying section represents the largest user of energy in the papermaking stage. In the middle of this section is the size press, which can apply coating to the paper. The size press must be placed so that the paper can continue drying after coating because the coating itself must dry as well. The next step is calendering, which involves a series of carefully spaced rollers that control the thickness and smoothness of the final paper. After calendering, the finished paper is wound on a large reel for storage and transportation (Klaas Jan Kramer, *et al*., 2009). A steam-filled dryer is a cost effective method to transfer heat into the sheet. The energy in steam has proven to cost less than a quarter of any other available method (Pauksta, 1998).

Figure 7: Drying process

SOURCE: www.dssmith.com

Non wood fibers

Currently, wood is by far the major raw material for the global pulp and paper industry. However, it is a relatively new raw material in papermaking. Historically, paper was made exclusively from non-wood plant fibers. While non-woods were originally used for papermaking, in the late seventeenth century wood became the predominant fiber source in Europe. The seemingly inexhaustible supply and versatility of wood were the major causes of this shift. Today, most modern pulp and paper enterprises rely on wood (Smook, 1992).

The term non-wood fiber encompasses a range of plants with widely differing characteristics. Non-wood fibers, also referred to as ‘‘alternate fibers’’, are non-woody cellulosic plant materials from which papermaking fibers can be extracted. Most non-wood plants are annual plants that develop full fiber potential in one growing season. There is a wide variety of non-wood plant fibers that can be used for papermaking (Touzinsky, 1993). Non-woods such as bagasse, wheat and rice straws, bamboo, and kenaf are being used in the manufacture of pulp and paper all over the world (Pande, H.; Roy, 1996).

Shortage of wood fibers

Due to the rising global demand for fibrous material, worldwide shortage of trees in many areas, and increasing environmental awareness, nonwoods fibers have become one of the important alternative sources of fibrous material for the 21st century. World demand for paper has increased at an average annual rate of 4.7% over the past 40 years. With the rapid growth of economies in the Asia-Pacific region and Eastern Europe, it is likely that similar growth in demand will continue in these regions for the foreseeable future. The existing wood resources in these regions may be inadequate to meet this growing demand for paper. In addition, logging is coming under increasing pressure from environmentalists concerned about habitat destruction and other longer-term impacts of forest harvesting. It is, therefore, necessary to consider alternative fiber sources to meet the possible shortfall of wood fiber for papermaking (Alireza Ashori, 2006).

Surplus of non wood fibers

The production of non-wood pulp mainly takes place in countries with a shortage of wood, such as China and India (Oinonen and Koskivirta, 1999). The utilization of non-wood fibers is an ethically sound way to produce pulp and paper compared to the clear-cutting of rain forests or primeval forests. The benefits of non-wood plants as a fiber resource are their fast annual growth and the smaller amount of lignin in them that binds their fibers together. Another benefit is that non-wood pulp can be produced at low temperatures with lower chemical charges. In addition, smaller mill sizes can be economically viable, giving a simplified process. Non-wood pulps are also more easily refined. Moreover, non-food applications can give additional income to the farmer from food crops or cattle production (Rousu *et al*., 2002; Kissinger *et al*., 2007; Rodríguez *et al*, 2007).

Non wood fiber as raw material

There is a growing interest in the use of non-wood such as annual plants, naturally growing plants and agricultural residues as a raw material for pulp and paper. Non-wood raw materials account for less than 10% of the total pulp and paper production worldwide (El-Sakhawy *et al*., 1996). This is made up of 44% straw, 18% bagasse, 14% reeds, 13% bamboo and 11% others.

Types of Agricultural Residues

There are numerous types of agricultural cropping residues. For pulp and paper purposes, only those with cellulose (fiber) content are of real interest. Corn stalks, leaves, and sheaths, wheat straw, rice straw, barley straw, oat straw, seed grass straw, and bagasse from sugar cane are all examples of agricultural cropping residues. When sugar cane is grown for the bagasse, it is called an intentional or on-purpose crop and is no longer considered to be a residue.

A. Wheat straw

Wheat is harvested across the United States and its straw is an ideal agricultural residue for use in paper and paper products. Its lignin content is comparable to hardwoods and it has one of the highest cellulose contents of all of the agricultural fibers. Wheat straw is clearly the most abundant nonwood plant material available for pulp and papermaking in

the United States. The amount of wheat straw available for pulp production varies depending on the type of wheat grown (Paul Patterson, *et al.,* 1995*).* The conversion figure tends to be in the range of 1.0-1.1 tons of straw produced per ton of grain. Wheat tends to have a much higher straw residue yield than barley. Straw papers are known to possess good printing qualities and are made from pulp requiring low energy relative to that required to process wood pulp. Technicians have found that wheat straw must be pulped under conditions of less energy and fewer chemicals than wood pulps to maximize pulp yields. To make the strongest product, papermakers will likely combine some stronger hardwood, kenaf, or hemp pulp with straw pulp.

B. Rice straw

Rice straw is another of the so-called "cereal straws," and contains a low concentration of lignin. Rice straw has a higher silica content (as high as 18 percent in "clean rice straw," and 19-24 percent including silica from field dust) (Jay Jeyasingam, 1985) than other cereal straws, which makes pulping somewhat more costly due to increased chemical recovery difficulties and costs. China currently has the greatest capacity for pulping rice straw, and to date there are no rice-straw pulp mills in the United States, although an engineer/entrepreneur named Al Wong is proposing to build one in California.

C. Corn waste

Iowa State University is experimenting with the manufacture of a stressed skin panel made from a number of different fibers, including corn stalks. Heartland Fibers, a midwest company, has also announced that it intends to produce pulp from cornstalks. Little information is available on the efficacy of using corn wastes, a problem which requires a remedy**.**

D. Cotton linters

Cotton linters - compared to woods and nonwoods - are loose fibers. Therefore, they can be easily transformed into pulp. Cotton linters represent a small morphological part of the cotton plant i.e. merely the seed hairs. If you take the rest of the plant i.e. the cotton stalks, you have then a whole plant consisting of stems, branches, leaves and cotton bolls. Egyptian cotton species are characterized by low content of cotton linters. Cotton

linters represent a costly agricultural waste. It possesses long fibers and is made mainly of cellulose characterized by high DP. It is used only for production of specialty paper, where permanence and durability are required. These are security paper, document paper, filter paper etc. (Yehia Fahmy, *et.al*., 2017).

E. Cotton stalks

Cotton stalks represent an important agricultural waste in Egypt. Here, the fibers i.e. the cells are held strongly together within the stem of the plant and are not loose as cotton linters. This product is called pulp and it is the intermediate raw material in papermaking. Technically this process depends almost upon dissolving the lignin that holds the cells together in the plants as in the tree trunk. Therefore, the process is also called delignification. Different plants contain different amounts of lignin. Lignin has different chemical structure in different plants and it varies in the ease in which it can be removed according to plant type. The chemical composition of the fibers defines and affects pulp ability i.e. the ease of pulping of the plant raw material. The ease of lignin removal is an important factor affecting pulp ability (Yehia Fahmy, *et.al*., 2017).

F. Bagasse

Bagasse is the fibrous residue which is remaining after sugarcane is crashed to extract its juice. By using agricultural residue rather than wood will add advantages of reducing deforestation. Sugarcane bagasse is particularly studied because it is one of the most important raw materials for paper pulp production in many countries (Khalsa Al-Sulaimani, Dr.Priy Brat Dwivedi, 2017). Bagasse is used for each of the four main paper categories, namely packaging and boxes, printing, writing and photocopier paper, tissues, and newsprint (Thomas J. Rainey, Geoff Covey, 2016). Research showed that sugarcane bagasse has lowest content of silica, 9.78% and highest content of carbon 90.22% and also contain 48-50% moisture, 48% fibre and 2-4% sugar. The fuel value of bagasse is mainly by virtue of its fiber content, which in turn contains 45% cellulose, 28% pentosans, 20% lignin and 5% sugar and 2% minerals (Salleh, Kasim &Saad, 2005).

Natural growing plants

Many fast growing annual and perennial plants, have been identified, cultivated and studied for their suitability as alternative source of raw material for paper industry (Nelson *et al*. 1966; Cunningham *et al*. 1970; Watson *et al*. 1976; Cunningham *et al*. 1978; Zarges *et al*. 1980; Mohanrao *et al*. 1982).

A. Cyperus papyrus

Cyperus papyrus, commonly called papyrus or paper plant, is a member of the sedge family (Cyperaceae). It is a monocot that is native to riverbanks and other wet soil areas in Egypt, Ethiopia, the Jordan River Valley, and other parts of the Mediterranean basin (Matt Burmeister, 2001)**.** Egyptians, using this plant, invented the first flexible writing support of vegetable origin (P.T. Nicholson and I. Shaw, 2000). Papyrus is not technically paper, since it is not made of felted fibers, but it shares with paper its vegetable origin, its composition mainly of cellulose, its form (rectangular sheet), its flexibility, its lightness and, above all, its function as writing support. Papyrus is made from pith, the naturally white inner part of the *Cyperus papyrus* stem, after the peeling and removal of the green and siliceous outer rind. The pith material is composed mainly of cellulose and some lignin. This pith was cut into strips, and then pressed down to form a sheet. The vegetable material was used raw, without refining. Papyrus was never sold in the form of a sheet, but always in the form of rolls of about 20 sheets joined together, which came in various heights, from 5 to 40 cm (H.G. Weidemann and G. Bayer, 1983).

B. Bamboo

Bamboo appears as an alternative source for pulp and paper industry, particularly in the tropical areas of the world. Bamboo is the vernacular or common term for members of a particular taxonomic group of large woody grasses. Bamboos encompass 1250 species within 75 genera, most of which are relatively fast-growing, attaining stand maturity within five years, but flowering infrequently. As an industrial raw material, bamboo has been used to produce both cellulosic fibers for paper and starch granules for saccharification and production or ethanol. In general, the D-cellulose content in bamboo is 40 to 50%, which is comparable with the reported D-cellulose contents of softwoods (40 to 52%) and hardwoods (38 to 56%)4 . Undoubtedly, bamboo is a potential alternative source of raw

material for dissolving pulp production because of long fiber (Susi Sugesty, Teddy Kardiansyah, Henggar Hardiani, 2015).

C. *Sabai grass (Eulaliopsis binata)*

Eulaliopsis binata is commonly known as 'Sabai grass', 'Bhabhar' or 'Golden grass' is characterized by its durability, strength and hardness. Sabai grass is second only to bamboo in importance as a raw material for pulp and paper manufacture in India. Sabai grass is a tuft perennial grass 2-5 ft high with erect, slender culms, shiny and wooly at the base. It is hardy to both frost and drought regions. It can grow on poor soils not subject to water logging. Sabai grass is harvested twice annually in August-September and November-December. The grass flowers during the cold weather and for the purpose of paper manufacture, the grass should be cut prior to or during the flowering stage. Sabai grass when carefully collected free from weeds and foreign matter forms an excellent material for the production of printing and medium quality writing paper. It is also suitable for straw-board (Dharm Dutt, *et.al.,* 2003).

D. Sisal (Agave sisalana)

Sisal is a nonwood leaf plant native to Mexico. It has successfully thrived in semi-arid regions of Brazil, Tanzania and Kenya. Fiber extracted from the leaves of the *Agave sisalana* plant and its hybrids can be used to produce high quality papermaking pulp. Sisal pulp has certain characteristics such as high tear resistance, high alpha cellulose content, high porosity, high bulk, high absorbency and high folding endurance which make sisal pulp suitable for many specialty papers. Also, because sisal pulp has physical properties superior to softwood kraft pulp, there may be opportunities to cost effectively replace softwood kraft with sisal pulp in commodity papers. For example, sisal pulp may be used as a reinforcing fiber in high recycle content papers, or its use may permit basis weight reductions while maintaining product quality. Markets for sisal pulp are established in the specialty paper sector; however, currently there are no markets established in the commodity paper sector (Robert W. Hurter, 2000).

E. Manila Hemp (Abaca)

Abaca is a bust fiber. The abaca fiber is extracted from the stalk of the plant. Abaca is also known as Manila hemp. This, however, is not the common hemp plant (*Cannabis sativa*), but is produced from the fibers of the *"Musa textilis"* a species of banana, and is called by Tagals *abáca*. The plant comes in great quantities from a almost every one of the Philippines, from Luzon to Mindanao.

Abaca fibers are extensively used to produce ropes, woven fabrics, tea bags, etc. It is also called biodegradable and sustainable fiber. Abaca is considered the strongest of natural fibers, being three times stronger than sisal fiber, and is far more resistant to saltwater decomposition than most of the vegetable fibers. Fibers are thin walled, uniform in width, and usually taper to a pointed end. The fibers appear stiff and have smooth walls if compared to other bast fibers, with fine, occasionally diagonal striations. The fiber are harvested every 3-8 months, after an initial growth period of 18-25 months, while the plants themselves live for up to 10 years.

Due to the fibers being resistant to salt water damage, the fibers are commonly used in ropes, lines, hawsers or nets used on ships. Other uses for the fibers include in rough papers, bagging, folders, handicrafts and rugs. A report from the Bureau of Agriculture of the Philippine Islands states that about 12,000 bales of abaca (Manila hemp) fiber are consumed monthly in making paper in Japan, and that this amount is likely to increase considerably if the market price for the fiber remains as low as at present (Peralta,1996).

Annual plants

Four species of fast growing annual plant viz. *Hibiscus sabdariffa, Crotalaria juncea, Tephrosia candida* and *Hibiscus cannabinus* and a variety of reed, *Neyraudia reynaudiana* were evaluated in the laboratory for their pulp and paper making properties. Data on proximate chemical analysis of raw materials, unbleached and bleached pulp properties, morphological properties of fibers and physical strength properties of paper sheets were evaluated (C. N. Saikia, T. Goswami, F. Ali, 1996)

A. Flax

Flax is grown extensively for making linen and linen-seed oil. The residual material after removal of the long fibers is known as flax strives. The strives contain about 45

percent cellulose (pure linen is more than 80 percent cellulose) and 13 percent lignin. The yield of flax seed straw per area of cultivation is low. The average pulping yield, varying between 25 and 60 percent depending on the cleanliness of the straw, is also considered to be on the low side. All these result in an extremely expensive pulp which can be used only in very high-priced end products. Such are cigarette paper, for which flax pulp is extensively used and other thin papers requiring high strength, such as banknote and airmail papers. It is estimated that something less than 100 000 tons of flax pulp are produced in the world. The use is expected to continue to increase although the very high price of the pulp will keep the quantities at a modest level. Flax strives on the other hand, as has been mentioned earlier, constitute an important raw material in the manufacture of particle board.

B. Kenaf

Kenaf is a plant that is similar to jute or hemp. It has a pithy stem surrounded by fibers. The fibers represent 20 to 25 percent of the dry weight of the plant. Mature kenaf plants can be 5 m tall. Historically, kenaf fibers first found use as cordage. Industry is exploring the use of kenaf in papermaking and nonwoven textiles. Kenaf fiber can be used to make letterhead quality paper. Whole kenaf stalks can be used to make newsprint grade paper (Lamahieu, *et al* 1991).

C. Gampi

Gampi is a relatively short fiber with faint surface markings. It is identifiable by the broad central portions that occur in many fibers. There are also immature, point ended, fibers that are often present in the pulp that stain yellow, and occasionally red. Fiber ends are found in many shapes, including, blunt, rounded, forked and others. There are many associated cells, which stain blue, that are a variety of shapes, including long rectangular and are often broken. The fibers are known to contain a natural muscilage that helps disperse the fibers, making the formation of thick sheets.

Growing as a small shrub, Gampi has been used for centuries in Japan as a source of high quality, fine paper. Gampi paper is famously known for its use as Torinoko paper. Torinoko translates to ‘bird’s child’ because of its semi-transparent, glossy, egg-like surface. The paper doesn’t bleed easily and is known to shrink and wrinkle with exposure

to water or heavy ink. These characteristic make it more appropriate for fine lines, such as with Kana Calligraphy, (Japanese syllabary) or in the transcription of Buddhist scriptures, or letters. Gampi papers are made in combination with other fibers to improve the working properties when large amounts of ink are used. Gampi paper is also used for Japanese style painting, luxurious sliding door or window covers (Travis Taylor, 2011).

D. Hemp

The hemp (*Cannabis sativa* L) is used as fiber for pulp and paper dates back more than 2,000 years (Van Roekel, G J, 1994)**.** Compared to wood pulp, hemp pulp offers a four to five times longer fiber, a significantly lower lignin fraction as well as a higher tear resistance and tensile strength. The advantage of hemp over cereal straw is a higher biomass yield per hectare of cultivation (Bawyer, 2001; Burczyk, *et al*,. 2009; Prade, *et al*., 2011; Consentino *et al*., 2013; Finnan and Styles, 2013; Jankauskiene and Gruzdiene, 2015) and lower content of mineral substances than in wheat straw.

The papermaking potential of bleached kraft pulps made from hemp stalks, hemp woody-core and hemp bast fibers was studied and compared with that of bleached birch pulp and pine kraft pulp. It has been shown that among the pulps from these raw materials, hemp stalk pulp has the most useful properties for papermaking. This pulp is characterised by a tensile index similar to birch pulp, a higher tear resistance and bulk than pine pulp, and a light scattering ability and opacity that is comparable with birch pulp after beating (Dariusz Danielewicz, Barbara Surma-Ślusarska, 2017).

E. Reeds

Phragmites australis, commonly known as reed, has been studied. Phragmites australis is a Perennial growing to 3.6m (11ft) by 3m (9ft) at a fast rate. It is an invasive plant commonly found near waterways and especially near construction sites, ditched marshes, roadside ditches, and other disturbed sites. Such a plentiful fiber is being used in other areas of the world for papermaking with loaded environmental significance. It has been characterized for chemical composition of the raw material by determining the ash content, α-cellulose, hemi-cellulose, lignin and their extractable in ethanol, 1% soda and hot water. *Phragmites australis* used as raw material to produce cellulosic pulps for the

manufacture of paper and or paperboard, because it has high content of cellulose (33-36%) and hemi-cellulose (20-22%) (Maria Dolores Gomez-Sanchez, *et al.*, 2017)

F. Jute

Jute is an annual plant which grows to the tune of about 1.5 million tons. Jute can be used as an alternative raw material if we can produce value added handmade paper from jute. Whole jute plant consists of stick (woody portion) and bark (fiber) in the ratio of 2.5:1. Stick contains high lignin and has short fiber length, hence pulp produced from whole jute plant shows higher tensile but low tear strength as compared to jute fiber pulp.

Jute containing cellulose like any other raw materials used for paper pulp, has been found to be an excellent raw material for making good quality pulp and paper. The technologies for making pulp and paper from whole jute as well as from jute fiber are successfully developed. Attempts are being made to make it more cost effective. Once the commercial viability improves further, whole jute could become a major source for "Tree free" pulp and paper. The stem of jute consists of two different fibrous components, both of which are suitable for quality paper making and is similar to softwood fibers. The bark fibers offer strength to the pulp while the shorter core fibers provide appreciable surface characteristics.

G. Sunn Hemp (Crotalaria juncea)

Sunn hemp is an annual plant grown mainly in India and Pakistan, and grows to a height of 2-3 m. Fibers are separated from the inner bark by retting, and are stronger when wet. Due to this property, the fibers are especially desirable for making fishing nets and ropes, and this is where most of the fiber supply goes. Old fishing nets are therefore one of the largest source of crotalaria fibers for papermaking (Ilvessalo-Pfaffli, 1995). The average fiber length of sunn hemp is 8 mm and its average fiber width is 0.03mm.

Problems with utilizing non wood fibers

Utilization of non wood plant fibers, although very popular, has certain inherent drawbacks. Majority of these problems are technological, and require research in this area to be more competitive with wood fibers.

Logistics and Assured supply

Advocates of non wood fibers cite the fact that they can be supplied from agricultural residues and annual fibers grown by a large number of individuals as amongst the greatest advantages of the non wood option. To the established paper industry this is perhaps its greatest disadvantage. The non wood fibers have the potential to provide raw material for paper production with reducing the exploitation of natural forest and need for tree plantations. The non wood fiber could provide farmers with an alternative or additional income and contribute to the rural economy.

In some parts of Asia, non wood fibers already contribute significantly to farm income. One of the most important issues for mill owners is guaranteeing an assured supply of consisting high quality raw materials. Probably the biggest constraint is the logistics of supplying a mill. It would be virtually impossible to supply large quantities of non wood fibers to a mill. With the exception of bamboo, non wood fibers are annual plants and can be harvested only during a few months of each year. Consequently, 9 months supply need to be stored in order to operate the mill continuously throughout the year. This problem can be addressed to some extent using a range of non wood fibers with different harvesting periods but a large amount of fibers still need to be stored (Sadawarte, 1995).

Environmental impact of fiber crops

There are potential pollution and disease problems associated with the growing of large areas of fiber crops although the extent of the problem depends on the individual crop and management practices. Most fiber crops are relatively hardy and disease resistant. Plantations that are not managed intensively managed might use herbicides before planting and apply fertilizer at intervals during the rotation. Input requirements and disease problems can be minimized by management practices. In some cases, insecticides and herbicides are also used when harvesting and storing crops (Hazard, *et al.*, 1988).

Paper making

The problems with using non-wood pulps for paper making include, slow drainage, a limit to the dryness on the press, poor wet strength, a tendency to blister when dried rapidly and high shrinkage problems (Sadawarte, 1995). The low drainability characteristics of non-wood fibers, causes runnability problems which decreases paper making productivity (Assumpcao, 1993). These problems can be reduced by using a

mixture of long and short fibers and keeping the short fibers component below 50% (Paavilainen, 1993). The runnability of straw pulp was improved by four ways, removing the fine fraction, blending in some long fibers, removing some of the hemi-cellulose or adding dewatering agents (Kuang, 1991).

Table 1: **Advantages and disadvantages of using non wood fibers for pulp and paper making**

STAGES OF CYCLE	ADVANTAGES	DISADVANTAGES
FIBER SUPPLY		
Based on annual or perennial fibers or agri-residues	Reduces need to exploit natural forest and for tree plantations.	May be problems associated with monocultures of annual fibers, for example, fertilizer and pesticide use, impact on crop rotations, biodiversity. This varies with fiber, area and management regime.
	Use of agri-residues in some cases makes use of what otherwise would be a waste product.	Use of agri-residues may reduce the fertility of the soil if some are not ploughed back in.
	Using agricultural lands for fibers in developed countries can contribute to reducing food surpluses.	Replacing food crops with fiber crops could be determined in areas where food security is a problem.
	Can support small scale mills, therefore a much lower level of capital investment is required. Small mills employ more people per ton of pulp than large ones.	Unlikely to be able to support large scale mills; therefore do not get the benefits of economies of scale.
	Can alter amount produced	Non wood fibers tend to be bulkier than wood which

	annually.	increases transport and storage costs.
	Less energy required to pulp fiber.	If a chemical pulping method is used, the lower amount of lignin results in less which cannot able to used as a fuel.
	Less energy and chemicals required to bleach fiber.	
PRODUCTION		
Lower proportion of lignin than wood.	Alternative pulping systems which reduce the need for chemical recovery may produce useful byproducts.	Alternative pulping systems tend to use more expensive.
Higher proportions of silica than wood makes inappropriate conventional chemical recovery systems.		Non wood fibers perceived to be high risk. Paper makers reluctant to make required changes to their machinery to enable them to use non wood pulp.
Industry is dominated by wood pulp producers and is conservative.		Speciality market is small.
	Niche market for high priced speciality paper for which certain non wood fibers have inherent advantages over wood.	
DEMAND		
	Niche market for ‘tree free’ products for which premiums are payable.	Size of tree free market is unclear as which the level of premiums may be paid.
	Markets for long fibers to strengthen recycled fibers.	Very difficult to compete in the mass market due to the establishment of the wood based industry with their large economics of scale and cost of non wood materials generally being higher than wood.

SOURCE: The potential of the non wood fiber paper sector, Sarah Roberts, December 1996.

Employment

Large modern mills give less employment opportunities than small mills (Fellegi and Judt, 1991). It has been estimated that a 20,000 ton per annum agri-pulp mill could provide 25 manufacturing jobs in Canada (Wong, 1994). In India Rao, 1989 estimates that in small mills, 53.5 people are employed for ever thousand tonnes of pulp production per year.

Demand and Markets

One difficulty in assessing the viability of using non wood fiber for paper production is a lack of information on the potential demand for paper products from these fibers. There are two distinct markets for pulp, the specialist paper market and the mass market. Some non wood fibers have inherent advantages for certain specialist application, such as for products requiring high tensile strength. Non wood fibers can fetch premium prices in these markets. One of the most profitable mills in India is Pudumjees Pulp and Paper Mill which produces speciality paper from bagasse, waste and wood pulp (Riddlestone and Desai, 1992). The profit in specialist market is small. In developing countries, the vast majority of non wood fiber pulp and paper products are produced for the mass market where they compete with wood products. There is also some non wood pulp costing less than wood pulp. In general, it appears that at present non wood products are more expensive than those made from wood (Hazard, *et al.*,1988).

Nutrients for algal growth

After the environmental parameters are fulfilled, microalgae require presence of nutrients in the water (or habitat) in order to grow. Microalgae have relatively simple nutritional demand, which make them an attractive package for commercial production. Carbon, nitrogen and phosphorous are the major nutrients for survival of microalgae. Among other nutrients some are macronutrients such as sulfur, potassium, calcium, magnesium and some are micronutrients, like manganese, copper, molybdenum, boron,

iron, zinc, chloride, nickel etc,. These trace elements have an important role in many enzymatic reactions and biosynthetic pathways. The requirement for nutrients may vary depending on the type of microalgae species (Grobbelaar, Johan U, 2005).

Algae as a raw material for papermaking

Ubiquitous distribution and fast growth of algae mark their easy availability as natural resources and possibility of harvesting all year round cuts down the costs involved in farm land cultivation or resource import or transport (Kaushik N, Biswas S, 2014). Another important point to consider is the lignin deprivation in algae, although some cases of lignin content in algae (intertidal red algae *Calliarthron cheilosporioides*) have been documented.

Conventional pulp production from wood as basic material is done through mechanical or chemical process. Mechanical method gives high yield of pulp (90%) but large amount of energy is wasted to mechanically remove lignin from wood (20-35% of wood is lignin) whereas in chemical method, yield of pulp is low (50%). Rice straw, oat/ sugarcane residue: bagasse is used as substitute for wood pulp but these also have 12-19% lignin. Hence, for development of paper pulp, most energy is wasted on lignin removal. Exploitation of well bonded algal pulp can yield paper that requires no artificial treatments for lignin removal. It was claimed that paper production from red sea weeds algae like Gelidium (Tronchin EM *et al.*, 2002) (compared to wood pulp) takes shorter time, lower cooking temperature and minimum chemical usage. Compared to wood fibers, algal fibers are finer, more uniform in length, smoother, also have absorbent properties.

Algae cellulose is different from terrestrial plant cellulose. Cellulose in plants is produced by rosettes of synthesizing enzymes extruding ribbons of cellulose strands in crystalline groups excluding water (Somerville C, 2006). The ribbons have less surface area whereas algae cellulose has many times more surface area to volume thus making a very different product. Plant cellulose has specific surface area of 1 square meter per gram while surface area of cellulose from algae Cladophora sp. can be up to 100 times larger because the cellulose is extruded as single strands (Marsin P and Tomasz J, 2005). Furthermore, cellulose from some genera of filamentous green algae exhibit particularly high degree of crystallinity and exhibit preponderance of I-alpha; cellulose as opposed to I-

beta cellulose, making them more thermodynamically reactive in comparison to cellulose derived from woody or plant biomass.

A. Gracilaria sp and *Eucheuma cottonii*

The two genus of red algae, called *Gracilaria* sp and *Eucheuma cottonii,* were simply processed to make pulps without use of any bleaching chemical agents. The potential use of pulps made of the red algae as raw materials for papermaking was mechanically studied by testing the sheets made of the red algae through a tensile test at a room temperature under 20 mm/min. Tensile properties of the proposed algae based paper sheets obtained under the constant rate are discussed. Tensile properties of the selected wood-based paper sheets obtained under the same condition are also presented in this paper. The results showed that pulps made of the red algae would be the alternative to those of the wood and other natural fibers as raw materials for papermaking (M. Nizar Machmud *et al.*,2013).

B. Gelidialian red algae,

Gelidialian red algae that contain rhizoidal filaments, except the family Gelidiellaceae were processed to make bleached pulps, which can be used as raw materials for papermaking. Red algae consist of rhizoidal filaments, cortical cells usually reddish in color, and medullary cells filled with mucilaginous carbohydrates. Red algae pulp consists of mostly rhizoidal filaments. Red algae pulp of high brightness can be produced by extracting mucilaginous carbohydrates after heating the algae in an aqueous medium and subsequently treating the extracted with bleaching chemicals. In this study, paper samples were prepared from bleached pulps obtained from two red algae species (*Gelidium amansii* and *Gelidium corneum*) and compared their properties to those of bleached wood chemical pulps. The yield of bleached red algae pulp from the selected wild red algae (*G. amansii* and *G. corneum*) was 8–11%, with a brightness over 80%. The hand sheets produced from red algae pulp had very high smoothness and opacity, which are essential properties for high-valued printing paper, when compared to those of wood pulp (Yung-Bum Seo *et al.*, 2009).

C. Cladophora green algae

Unlike brown and red algae, which are harvested and processed on a large industrial scale, green algae so far have found only limited industrial use. However, polluting Cladophora green algae represent an unexploited, renewable source of highly crystalline cellulose material that features highly beneficial properties that could be used in various industrial applications. Finding high-tech, niche-product applications for Cladophora cellulose will clearly raise the awareness about the unique properties of this material and may alleviate serious environmental problems associated with seasonal algal blooms. Cladophora algae blooms are seasonal and sensitive to changes in light availability (Higgins, S. N *et al*., 2008) However, Cladophora algae rapidly adapt to changes in the temperature of the surrounding environment, and therefore, their photosynthesis and respiration rates are largely unaffected by such fluctuations. Cladophora green algae prefer large solid substrates with a high roughness and porosity for proliferation (Bergey E. A, 2008). For instance, the probability of Cladophora algae eutrophication is higher in areas with large stones with rugged surfaces, for example, on the concrete blocks of peers, whereas small stones with smooth surfaces are less attractive.

D. Ulva sp.

Algae belonging to the genus *Ulva* are very common worldwide, fast colonizers. *Ulva sp.* has been studied for different application. One of the applications was making paper with this algae. Monegato and Nicolucci at 1995 invented a method to manufacture from seaweed mainly *Ulva rigida,* along with bleached wood pulp. Later, You and Park at 2009 invented a method to manufacture paper from Rodophyta algae. *Ulva sp.* had less cellulose content when compared with vascular plants, but the low content of undesired compounds have given to remove prior to extraction, it can be concluded that its chemical composition provides ease to the manufacturing of cellulosic products (Ana Moral *et al.*, 2014).

Fibres for paper

Most plant cell walls consist of cellulose, but in algae cell coverings are very diverse. Some algae species have intracellular walls, or scaly cell walls made of deposits of calcium carbonate or silica, but most algae derive structural strength from continuous

sulphated polysaccharides in marine algae; other possibilities being cellulose, carrageenan, alginate and chitin (Okuda 2002).

Cellulose-containing algae can potentially be used as a renewable feedstock for paper production as the strong green colour of algae is more difficult to bleach than wood fibres but, although algae are generally known for their low cellulose and hemicellulose content, there are a few examples of research into the use of algae as a non-wood fibre source. Ververis *et al.*, 2007 used a mix of algae taken from a municipal waste water treatment as 10% of the pulp mix, resulting in a significant increase in the mechanical paper strength and a decrease in paper brightness. The best result of pure algae-paper approached standard paper quality, showing lower bursting, tearing and folding strengths. Mixing with softwood pulp improved the paper to Kraft quality (Chao, Su *et al*. 1999; Chao, Su *et al*. 2000; 2005). This algae is filamentous (forms long threads) and is therefore much easier and cheaper to harvest than unicellular algae. Another benefit is the salt tolerance of *Rhizoclonium*, ranging from 1.0 to 3.3 % salt, with an optimum at 2.0% salt (seawater averages 3.4% salt). At this optimum, most naturally occurring freshwater algae will not be able to grow. *Chaetomorphalinum* and *C. Melagonium* have similar cellulose contents (Chao, Su *et al*. 1999), while *Vaucheria* species can contain about 90% cellulose in their cell wall (Parker, Fogg *et al*. 1963) in (Okuda 2002).

Biologically different from algae and seaweed, but similar in cultivation and processing are certain aquatic plants. These may also have high productivities and may be grown on waste streams, and since they are closer to land plants, have high fibre contents. Joedodibroto, 1983 has investigated several aquatic plants and concluded that all three weeds produced moderate quality paper pulp. Water hyacinth gave good folding and tearing resistance, but the processing of material from this plant was rather difficult. Other investigators reported that paper from 75% water hyacinth pulp and 25% bamboo pulp gave a high strength and also good greaseproof properties to the paper (Goswami and Saikia,1994).

There is some promising research on the utilization of aquatic biomass for paper pulp, a development that deserves future attention, from economical, renewable and quality

points of view. However this concept has not moved beyond the research stage yet and it is unclear when it will be commercialised.

World paper consumption was about 300 million tons in 1996/97 and is expected to rise above 400 million tons by the year 2010 (Hurter and Riccio, 1998). In view of the shortage of conventional raw materials for pulping and the increasing demand for paper products, new raw materials for pulp production such as non-wood fibers are being investigated worldwide (Ververis *et al*., 2004). Some of the new materials are either filamentous algae that can be used as the main raw material for papermaking (Kiran *et al*., 1980; Sakai *et al*., 1996; Chao *et al*., 2000) or algal biomass used as a supplement in softwood or hardwood pulps (Nicolucci *et al*., 1994).

The major problems in producing paper from filamentous algae are (i) the isolation of certain species and growth of biomass in culture solutions inevitably increase the process cost and (ii) the relatively poor mechanical strength of the algal pulps (Chao *et al*., 2000). On the other hand, mixing algal biomass with conventional paper pulp seems a more promising method for exploiting algae from eutrophic waters (Nicolucci *et al*., 1994).

The use of Red algae for producing raw materials for papermaking with regard to their physical components which contains large amounts of mucilaginous materials such as agar or carrageenan, which can be easily extracted with hot water, and small amounts of solid materials, which are endofibers. After the extraction of the mucilaginous materials, the remaining material mostly consists of endofibers (also known as rhizoidal filaments, rhizine, internal filaments, and hypha (Lee *et al*., 2003), which are then bleached to make bleached red algae pulp. These endofibers could be used as a new type of raw material for papermaking, which would be more abundant worldwide than wood fibers. The average growing rate of some red algae in the sea is around 3–10% per day (dry weight) during the growing season (Gel-Or *et al*., 2004; Ohno *et al*., 1996; Felicini *et al*., 1994), and red algae grow under the sea surface worldwide except in the arctic areas. There is no quantitative limit on the supply of endofibers or red algae pulp as long as investment for their cultivation in the sea and the necessary processing facilities are available.

Alginates in Paper Industry:

Alginates partially complexes with calcium (such as forming a loose gel) and when mixed with starch was proposed to get high water retention in paper coating (Joyce, M. and Gilbert, S.A.) thus finds application in paper industry for supplementing wood pulp.

Quality of paper:

Testing the quality of the paper by physical and optical properties that are Basis weight, bulk, bursting strength, tearing strength, tensil strength, breaking length, smoothness, porosity and brightness.

Basis weight:

It is the "weight of paper per unit area "It is expressed as grams / sq. meter.It influences all paper properties. According to end use, gsm requirement varies.

Bulk:

It is the volume of paper per unit weight and is reciprocal of density. It's unit is cc/g.Bulk=Thickness in Microns / Basis wt in gsm**.** This influences all other properties**.** High bulk gives good resistance and superior printing quality. Bulk decreases with improved smoothness**.** Low Bulk paper has low opacity**.** To maintain Bulk, proper furnish mix, low refining, low calendering and low ash content are being maintained**.**

Bursting strength:

Bursting strength is defined as the hydrostatic pressure in Kilo-Pascal, or Kilo-Newton per square meter required to produce rupture of the material when the pressure is increased at a controlled constant rate through a rubber diaphragm.To compare at various gsm of paper it is expressed as Burst factor.

Burst factor = (Bursting Strength X 10.2)/gsm

This is an important property for packing grade paper**.** By proper refining the stock and by adding starch this property is being maintained.

Tearing strength:

Tear is the force required to tear a single sheet of paper through a specified distance and is expressed as Tear Factor.

Tear Factor = (Tearing Strength X10.2)/ gsm**.**

This is an important directional property for Tissue, Wrapping, Packing and Specifically for printing paper in reel form. Proper furnish mix and refining ensures required tearing strength.

Tensile strength:

Tensile strength is an indicative of the serviceability of many papers, such as web printing, wrapping, bag and gummed tape and creped papers such as cable wrapping, tissue and toweling, which are subjected to direct tensile stress . The tensile strength tells how well the paper will resist breaking during a process such as printing. Tensile strength measures the force required to break a standard width strip (15 mm).

Breaking Length:

Breaking length which is a strength to weight ratio which indicates the length of a strip of paper required to cause the strip to break under its own weight when hang freely (OR)The breaking length of a paper is the length of a uniform strip that is just sufficient to break under its own weight when suspended at one end. This is the length of a strip of paper expressed in meters, which would break on its own weight when suspended vertically.

Tensile strength of paper is always greater in the machine direction than in the cross direction because of the greater alignment of fibers in the machine direction. The ratio between machine and cross direction tensile strengths is an indication of the squareness of the sheet. By proper refining of stock and optimising the ash content this property is controlled.

Smoothness:

It is measured by the amount of air escaping along the surface of the paper under a specified weight and expressed as millilitre / minute in Bendtsen. Smoothness is concerned with the surface contour or mechanical perfection of the paper surface. An ideally smooth surface is one in which all the surface elements lie in one plane. The smoothness of a real surface such as that of paper can thus be easily defined as the closeness of its surface to the plane surface.

Smoothness means freedom from lumps, wire and felt marks, fuzziness, foreign matters, inter fiber voids, crush, cockles, mechanical damage (scabs, press and calender cuts) and incompressibility and other gross surface perfections. Generally rough papers require more ink in printing causing excessive show through and poor halftone dot formation. Because of more ink carried on the plate, the ink mileage is reduced.

Porosity:

Porosity is the rate of passage of air through the paper under the influence of a difference in pressure. Therefore, porosity indicates the structural characteristics of the paper and in particular, the diameter and spacing of the capillary air passage. Porosity is inversely proportional to density. Porosity is a property of direct importance in writing and printing papers since it is a factor in the absorption of inks.Lesser the porosity of paper, printing ink consumption will be less.

Brightness:

It is the percentage of blue light reflected of a sample measured at an effective wavelength of 457nm and it is distributed throughout the spectral range of 400-500 nanometers. Brightness is also well suitable for measuring the permanence of paper, since the change in the color of paper on aging or thermal degradation is greatest in the blue and violet regions of the spectrum where brightness is measured.

Natural Additives to Algal Pulp in Paper-Making

Improvement of paper quality need pulp improvisations relative to the application of the paper. This involves additives to the algal pulp prior to the cooking process. Rice husk (RH) or hull is the outermost layer of the paddy grain accounting for 20% of paddy weight having good absorbent properties. High silica contents of rice husk (20-50 %) increases its Pozzolanic effect that determines cementations' properties responsible for increasing the rate at which a material gains strength (Jauberthie R *et al.,* 2000). Asia produces about 770 million tons of husks annually obtained from milling of rice grains which can be put to low cost productive usage as algal pulp additive. Saw dust does not have a proper texture and hence is not suitable for writing/painting paper but can e used in making of thick material like egg cartons. The presence of proteins and chitin in algae as

factors responsible for significant improvement in mechanical properties of paper from algae compared to conventional pulp (Ververis *et al*., 2006). Although, brightness was adversely affected by chlorophyll in algae; the cost of raw materials was 45% lower than that of conventional pulp. Addition of never-dried Rhodophyta algae fibers to CP provided pulp sheets with improved tensile and burst strength properties, with minimal deterioration in Canadian standard freeness (>200 ml) (Kaisha MJK, 1992).

Advantages of using algae as raw material for papermaking

Using algae as paper pulp has certain extra advantages. For example:

- An algae is fast growing and annual crops could be utilized causing no damage to the existing flora thus saving a large part of perennial biomass.
- Since algae have great roles in carbon sequestration, it helps to reduce the carbon dioxide levels of atmosphere. When paper making is the aspect to focus on, the tough points with algae are pigment and water removal: bleaching and drying; production and energy costs related to pilot or large scale production (which have not been tested), financing and market value. Paper made from pure algae fiber suffers from poor bursting strength, tearing strength and folding strengths (Chao KP, Yu CS and Chung SC, 2000). Thus, mixing of algal pulp with other raw materials like softwood fibers needs to be exploited to improve paper properties (comparable to Kraft paper).

Applications

Algae have come up as a valuable raw material for paper besides bioplastics and furniture building in the US and Japan. For designers Jacob Douenias and Ethan Frier, Spirulina algae also have a role in our homes as lighting and furniture, producing food, fuel, heat and light. Designers Jonas Edvard and Nikolaj Steenfatt have used a new material made from Danish brown Fucus (Stegenga H, Bolton JJ and Anderson RJ, 1997) seaweed and paper to create a chair and a collection of pendant lamps. Thus, algae as a raw material have a long way to go. The continuous lumbering of forests to meet the ever increasing needs of the paper industry is posing an international threat to our environment, partly contributing to global warming. Thus, switch to non-wood material like algae is an urgent issue to consider for the paper industry.

MATERIALS AND METHODS

3.1 Sample collection:

Algal samples both Microalgae and Macroalgae were collected in order to identify characterize and take them for pulp supplement. Macroalgae was collected from the marine areas whereas the microalgal samples were collected from fresh water bodies, Kanyakumari, and Effulent treatment plants.

3.1.1 Microalgae:

The microalgae samples were collected from different locations. The sampling locations have been assigned as Effluent treatment plants (ETP), fresh water bodies, and Kanyakumari where the algae grow as a biofilm over the surface of water. The samples were collected from the ETP of PSG College of Arts & Science, Coimbatore. The samples were collected from different tanks of the effluent treatment plants. Samples from each tank whose biofilm morphology differs were collected. Twenty different microalgae samples were collected.

3.1.2 Macroalgae:

The macroalgae samples were collected from the marine areas. The macroalgae samples were collected from the coastal waters of Tharivaikadu, Thoothukudi District. Five different marine macroalgae were collected.

3.2 Morphological characterization:

The collected samples were identified and characterized based on their morphological/microscopic features.

3.2.1 Microalgae:

The microalgae samples were collected from three different locations. The sampling locations have been assigned as effluent treatment plants (ETP) where the algae grow as a biofilm over the surface of water. The samples were collected from the ETP of PSG College of Arts & Science, Coimbatore. The samples were collected from different tanks of the effluent treatment plants such as the settling tank, aeration tank, anaerobic tank and clarifier tank. Two samples from each tank whose biofilm morphology differs were collected. Also samples were collected from fresh water and marine sources and taken for identification asnalysis.

3.2.1.1 Wet Mount Technique:

The samples were thinly sliced using a dissection needle into individual thread like substance and then placed onto petri dish cover containing distilled water. Then these individual threads were picked up and placed onto a clean glass slide and cover slip was placed over it. Then these slides were focused under the microscope primarily under the 10 x objective and then under 40 x objectives. The morphological features observed were documented.

3.2.2 Macroalgae:

The macroalgae samples were characterized morphologically based on their macroscopic features such as thalli, colour etc.

3.3 Cultural Characteristics:

Culturing of the algal samples was done on the BG-11 media. Only the microalgae samples were cultivated under the laboratory conditions. The most commonly found organisms from the different sampling locations were selected and inoculated into the BG-11 media.

3.3.1 Media preparation:

BG 11 Medium:

STOCK SOLUTIONS:

STOCK - 1:		
Disodium magnesium EDTA	-	0.1g
Ferric ammonium citrate	-	0.6g
Citric acid monohydrate	-	0.6g
Calcium chloride dehydrate	-	3.6g
STOCK - 2:		
Magnesium sulphate heptahydrate	-	7.5g
STOCK - 3:		
Di-potassium hydrogen phosphate	-	3.05g
Di-potassium hydrogen phosphate trihydrate	-	4.0g
STOCK – 4:		
Boric acid	-	2.86g
Manganese chloride septahydrate	-	1.81g

Zinc sulphate septahydrate	-	0.222g
Copper sulphate septahydrate	-	0.079g
Cobalt chloride hexahydrate	-	0.050g
Sodium molybdate dehydrate	-	0.391g
	(OR)	
Molybdate	-	0.018g

All the stock solutions were prepared for 1 liter. They were all sterilized at 121°C for 15 min at 15lbs/inch^2 pressure.

WORKING MIDIUM:

STOCK - 1	-	10ml
STOCK - 2	-	10ml
STOCK - 3	-	10ml
Sodium carbonate	-	0.02g
STOCK - 5	-	1.0ml
Sodium nitrate	-	1.5g

All stocks are combined and pH is adjusted to 7.5 (1.0N HCI). The medium is autoclaved and then cooled.

3.3.2 Culturing:

100ml of the medium in 250ml flask were prepared for each sampling locations. Then the samples were inoculated into the medium. They were then incubated at 28ºC in algal chambers where the light and dark reactions were maintained using artificial light.

3.3.3 Cultural characteristics:

The cultured samples were observed for morphological features after 3-4 weeks of incubation under the microscopic. 100µl of the sample was taken using micropipette and placed on a slide. Cover slip was placed over it and viewed. The morphological features observed were recorded.

3.4 Confirmation:

The observed morphological features of both microalgae as well as macroalgae were confirmed using the reference articles for each. (Fritsch"Structure and Function of Algae") and internet sources for their morphological features.

3.5 Biochemical analysis:

The reducing sugar, glucose, cellulose, hemicelluloses, lignin, ash and lipid contents were determined. Twenty five samples were chosen for the biochemical analysis in which five samples are macroalgae (*Gracilaria sp,, Sargassum sp, Chaetomorpha sp, Ulva sp, Kappaphycus sp,*) and twenty samples are microalgae (morphologically different biofilm collected from different locations).

3.5.1 Sample treatment:

Twenty five samples of air dried, ground (0.5 mm) algal biomass (0.7 g each) were boiled with 5mL of 72% v/v H_2SO_4 solution for 4.5 hours in order to hydrolyze the cellulose and hemicellulose. The suspension remaining after the above treatment was filtered through a crucible and the solid residue dried at 105ºC for 24 hours. The suspension was then used for the estimation of glucose, reducing sugar, cellulose and hemicellulose. The solid residue was used for the determination of lignin estimation. The filtrate from the H_2SO_4 treatment that contained the sugars released from cellulose and hemicellulose was thoroughly stirred and homogenized.

3.5.2 Reducing sugar: (Miller *et al.*, 1959*)*

The reducing sugar content of the 25 samples was analyzed using 3, 5 dinitrosalicylic acid (DNSA) (Miller *et al.*, 1959*)* method.

Reagent preparation:

3, 5 dinitrosalicylic acid (Aldrich)	-	10.0g
Na_2SO_3 (Ajax Finechem)	-	0.5g
Na-k tratrate (APS Finechem)	-	182.0g
NaOH (Merck)	-	10.0g
Phenol (Merck)	-	2.0g

Deionised water 1000ml

NaOH 10g are added into 700ml of deionised water and mixed in order to add the 300g Na-K tratrate. When the solution is dissolved, 3, 5 dinitrosalicylic acid 10g is then added and continuously stirred. After that the 0.5g of Na_2SO_3 and 2.0g of phenol are dissolved, respectively. Finally the volume is adjusted to 1000ml by deionised water and kept away from light.

Procedure:

The samples 0.5ml was mixed with 10ml of 80% ethanol. The samples were mixed thoroughly and then add 3ml of alcoholic extract with 3ml of DNS reagent. The mixture was boiled for 5 minutes. Add 1ml of Na-K tartrate in warm condition. The sample was cooled down by immersing the sample tube into cold water immediately. The mixture was mixed well, and the absorbance was measured at 570 nm is converted to reducing sugar concentration in comparison with standard curve.

3.5.3 Glucose estimation:

The glucose content of the 25 samples was analyzed using Anthrone method.

Reagent preparation:

0.2g of anthrone in 95% of sulphuric acid (ice cold) was added

Stock standard: 100mg of glucose in 100ml of distilled water.

Working standard: 10ml of stock solution was made upto 100ml of distilled water in a standard flask**.** This consists of 100µg per ml.

Procedure:

The working standard was taken and added to five test tubes each containing 0.2, 0.4, 0.6, 0.8 and 1ml of the standard. This consists of 20 to 100µg of glucose. To each of the tubes distilled water was added and made up to 1ml. 1ml of distilled water was taken as blank. The extracted samples were taken in test tube (0.5ml) and were made up to 1 ml. To all the test tubes 4ml of ice cold anthrone reagent was added and mixed well. The tubes were boiled in water bath for 8 minutes and then cooled down. The green colour formed was read at 620 nm in a UV spectrophotometer and the values were noted down. The standard graph was plotted using the standard glucose values and using the plotted graph unknown values were determined.

3.5.4 Cellulose estimation: (Miller *et al.,* 1959)

The glucose concentration estimated using Miller *et al.,* 1959 method was used to determine the cellulose concentration.

%w/w cellulose content = $0.9/0.96 \times C_1 \times (V/M) \times \alpha \times 100$

0.9 is the coefficient that results from the molecular weight ratio of the polymer and the monomer hexose. The saccharification yield was taken as 0.96, C_1 as the glucose concentration (g/L), V the total volume of sugar solution (L), M the dry weight of the algal biomass sample (g) and α the dilution of the sample.

3.5.5. Hemicellulose estimation: (Miller *et al*., 1959)

The glucose concentration and the reducing sugar concentration estimated using Miller *et al*., 1959 and Anthrone method respectively was used to estimate the hemicelluloses content.

% w/w hemicellulose content = 0.88/0.93 x C_2-C_1 x (V/M) x α x 100

0.88 is the coefficient that results from the molecular weight ratio of the polymer and the monomer pentose, 0.93 is the saccharification yield of xylane to xylose, $C_2$is thedetermined reducing sugars concentration (g/L) from the DNS method, C_1 the glucose concentration (g/L) from above, V the total volume of sugar solution (L), M the dry weight of the algal biomass sample (g) and α the dilution of the sample.

3.5.6 Lignin and Ash content: Ververis *et al.*, 2004

The solid residue obtained after treatment of sample was dried at 105°C for 24 hours and weight (W1). The residue was then transferred to a pre-weighed dry porcelain crucible and heated at 600°C for 5 hours. After cooling down, it was weighed (W2) and ash content (%) was determined. Acid insoluble lignin was then calculated by the difference (W1-W2)

3.5.7 Lipid: EG Bligh, WJ Dyer (1959)

Fourteen samples are choosen for the extraction lipid in which five samples are macroalgae (*Gracilaria sp, Sargassum sp, Chaetomorpha sp, Ulva sp, Kappaphycus sp*) and ten samples are microalgae. The extraction of lipid by EG Bligh, WJ Dyer (1959)

Procedure

0.2gm of sample was mixed with 5ml of Chloroform and Methanol (1:2, v/v) in a capped glass tube. Then it was placed in a room temperature. After 1hour add 2ml of chloroform and 3.6ml of distilled water and vortex vigorously for few minutes. Then centrifuge the suspension for 1000 rpm for 5 minutes and the organic phase is pipetted out in a sterile screw cap tube. Add same amount of chloroform is used to replace the organic phase pipetted out. Solvent evaporate is by keeping it in a water bath for 4-5 hours.finally the lipid extract is collected.

3.6 Pigment extraction: Dere *et.al*., (1998) and Yoshii *et.al*., (2004)

Ten samples were choosen for the pigment extraction (*Oscillatoria sp, Oscillatoria brevis, Microcystis sp, Oscillatoria sp, Phormidium sp, Nostoc sp, Microcystis sp., Microchaete sp.,, Diatom, Nostoc sp.,)* by using method of Dere*et.al*., (1998) and Yoshii *et al*., (2004).

3.6.1 Procedure:

Sample preparation:

1gm of samples were homogenized using mortar and pestle and made into fine paste by adding 10ml (50:50) mixture of diethyl ether with acetone. The filtrate from well crushed paste was transferred into conical flask through a funnel.

Column preparation and packaging:

Glass wool was placed at the bottom of the column and the column was mounted on the stand. 25gm of fresh silica gel was mixed with 100ml of diethyl ether and it was stirred well to make slurry of silica. The slurry was poured into the column. The conical was placed below the mounted column and the excess solvent (diethyl ether) was dried out.

Loading of sample on to the column:

The prepared sample extract was transferred into the packed silica gel column.

Elution using with diethyl ether:

The column was continuously filled with diethyl ether till the yellow colour β-carotene was completely eluted out. The eluted β-carotene was collected in a conical flask and stored separately.

Elution with acetone:

After collecting the yellow pigment, the column was filled with acetone. Another flask was placed below the mounted column to collect the green pigment moving down to the column. The eluted green colour Chlorophyll pigment was collected in a conical flask and stored separately.

Spectrophotometric analysis of Pigments:

For Chlorophyll a, Chlorophyll b and total Carotenoids calculation the supernatants were read at the absorbances of 470, 645 and 662 nm respectively by using spectrophotometer. The pigments were calculated by the following formula:

$Ca = 11.75 \times A662 - 2.350 \times A645$

$Cb = 18.61 \times A645 - 3.960 \times A662$

$CX+C = (1000 \times A470 - 2.270 \times Ca - 81.4 \times Cb) \div 227$

Ca = Chlorophyll a

Cb = Chlorophyll b

CX+C = Total carotenoids A = Absorbance

3.7 Application:

The application side deals with handmade paper making using the collected algal samples as pulp supplements. Microalgae were tested as supplements separately to determine their efficiency in paper making. The samples are used as such and without any pre-treatment for extraction of the cellulostic or other biochemically relevant products that are responsible for providing considerable strength to paper.

3.7.1 Apparatus for paper making:

The handmade paper making apparatus consist of a mesh that accounts for the size of A4 size sheet, a frame that could hold the four sides of the mesh and three plywoods of unique thickness, length and breadth. Water soaking cloth was cut down to the size of the plywood. Plastic tray to pour the slurry and it should be large enough to dip the mesh vertically as well as horizontally. Blender to prepare pulp for paper making.

3.7.2 Sample preparation:

3.7.2.1. ETP pulp:

ETP pulp was obtained from paper mill-Seshasayee Paper and Boards limited, Pallipalayam. ETP pulp was prepared by adding 40gm of ETP pulp in 500ml of distilled water. Then the pulp was poured into the tub and the mixture was mixed thoroughly.

3.7.2.2 Microalgae:

The microalgae samples were dried (shallow drying) and then it was cleared of all the debris material. Then the algal material was separated out as thin thread like forms and was grounded in blender. Then water was poured and its level of dispersion was compared with that of the blended ETP pulp. The level of dispersion of algae was made on par with of ETP pulp by either adding water to dilute it or by adding more algae to increase the slurry thickness. Then they were used for the paper making procedure.

3.7.3 Handmade paper making:

3.7.3.1 Control paper:

The mixture is poured into tub (ETP pulp) and the process is carried out till the slurry is about 4 inches. To make thin paper more water is needed. Scoop up the pulp into mesh and frame from the tub. Frames are lifted up and water is drained. Shake gently to distribute the fibers freely. The frame was separated and the mesh is layed upside down and soaking cloth is placed on the mesh. Then the plywood is placed above this cloth. Gently hold the plywood and turn the mesh. The mesh was removed and another cloth is placed above the wet pulp. Then another plywood is placed above this cloth and press the plywood for few minutes to remove the water from the pulp. The steps are repeated by changing the cloth until there was no more water to be soaked manually. Paper sheets were dried for ½ to 1 day.

3.7.3.2 Microalgae as supplement to pulp:

The microalage pulp that was prepared was added as supplements to paper pulp in the concentration of 10-50%. The ETP pulp concentration was decreased respectively with respect to the increasing algae pulp concentration. Then the similar steps as of the control paper making were followed. The resulting paper quality was documented.

3.7.3.3 Quality of paper:

Testing the quality of the paper by physical and optical properties: Basis weight, bulk, brusting strength, tearing strength, tensil strength, breaking length, smoothness, porosity, brightness.

3.9.1 Basis weight:

It is the "weight of paper per unit area". It is expressed as grams / sq. meter. It influences all paper properties.

3.9.2 Bulk:

It is the volume of paper per unit weight and is reciprocal of density. It unit is cc/g. High bulk gives good resistance and superior printing quality. Bulk decreases with improved smoothness**.** Low Bulk paper has low opacity**.**

3.9.3 Bursting strength:

Bursting strength is defined as the hydrostatic pressure in Kilo-Pascal, or Kilo-Newton per square meter required to produce rupture of the material.

Burst factor = (Bursting Strength X 10.2)/gsm

This is an important property for packing grade paper**.** By proper refining the stock and by adding starch this property is being maintained.

3.9.4 Tearing strength:

Tear is the force required to tear a single sheet of paper through a specified distance and is expressed as Tear Factor.

Tear Factor = (Tearing Strength X10.2)/ gsm**.**

This is an important directional property for Tissue, Wrapping, Packing and Specifically for printing paper in reel form. Proper furnish mix and refining ensures required tearing strength.

3.9.5 Tensile strength:

Tensile strength is an indicative of the serviceability of many papers, such as web printing, wrapping, bag and gummed tape and creped papers such as cable wrapping, tissue and toweling, which are subjected to direct tensile stress .

3.9.6 Breaking Length:

Breaking length which is a strength to weight ratio which indicates the length of a strip of paper required to cause the strip to break under its own weight when hang freely (OR)The breaking length of a paper is the length of a uniform strip that is just sufficient to break under its own weight when suspended at one end. This is the length of a strip of paper expressed in meters, which would break on its own weight when suspended vertically.

3.9.7 Smoothness:

It is measured by the amount of air escaping along the surface of the paper under a specified weight and expressed as millilitre / minute in Bendtsen. Smoothness is concerned with the surface contour or mechanical perfection of the paper surface. Smoothness means freedom from lumps, wire and felt marks, fuzziness, foreign matters, inter fiber voids, crush, cockles, mechanical damage (scabs, press and calender cuts) and incompressibility and other gross surface perfections.

3.9.8 Porosity:

Porosity is the rate of passage of air through the paper under the influence of a difference in pressure. Porosity is inversely proportional to density. Porosity is a property of direct importance in writing and printing papers since it is a factor in the absorption of inks.Lesser the porosity of paper, printing ink consumption will be less.

3.9.9 Brightness:

It is the percentage of blue light reflected of a sample measured at an effective wavelength of 457nm and it is distributed throughout the spectral range of 400-500 nanometers. Brightness is also well suitable for measuring the permanence of paper, since the change in the color of paper on aging or thermal degradation is greatest in the blue and violet regions of the spectrum where brightness is measured.

Second Year Project Work Methodology:

Algal samples were collected in order to identify, characterize and take them for pulp supplement. The bulk sample of algae was collected from water tank in the area of Bhavanisagar near Sathyamangalam. The sample was collected in a time interval of 15 days.

3.1.2. Cotton

The three different categories of waste cotton were collected from the cotton mill. The categories were based on their fiber length. Cotton is used as additive material for papermaking, due to its rich cellulosic content.

3.1.3. Humus

The waste leaves were collected from PSG College of Arts and Science, Coimbatore it is soaked in water and stored for pulp preparation.

3.2. Morphological characterization:

The collected samples were identified and characterized based on their morphological/microscopic features.

3.2.1. Wet mount techniques

The algae were observed under microscope using unstained wet mount techniques. The samples were thinly sliced using a dissection needle into individual thread like substance and then placed onto Petri dish cover containing distilled water. Then these individual threads were picked up and placed onto a clean glass slide and cover slip was placed over it. Then the slides were viewed under microscope at the magnification of 10X, 40X and 100X. The morphological features observed were documented.

3.2.2. Culturing

Culturing of the algal samples was done on the BG-11 media. Only the microalgae samples were cultivated under the laboratory conditions. The collected algal sample was inoculated into the BG-11 media.

100ml of the medium in 250ml flask were prepared. Then the samples were inoculated into the medium. They were then incubated at 28ºC in algal chambers where the light and dark reactions were maintained using artificial light.

3.2.3. Cultural characteristics

The cultured samples were observed for morphological features after 3-4 weeks of incubation under the microscopic. 100µl of the sample was taken using micropipette and placed on a slide. Cover slip was placed over it and viewed under the microscope. The morphological features were observed.

3.3. Biochemical analysis for algae and cotton

The reducing sugar, glucose, cellulose, hemicelluloses and lignin content were determined.

3.3.1. Estimation of Reducing Sugar by Dinitrosalicylic Acid Method

For sugar estimation, the dinitrosalicylic acid method is the simple, sensitive and adoptable during handling of a large number of samples at a time. Weigh 100 mg of the cotton algal sample and extract the sugars with hot 80% ethanol twice (5ml each time). Collect the supernatant and evaporate it by keeping it on a water bath at 80°C. Add 10ml water and dissolve the sugars. Pipette out 0.5 to 3 ml of the extract in test tubes and equalize the volume to 3 ml with water in all the tubes. Add 3 ml of DNS reagent. Heat the contents in a boiling water bath for 5 min. When the contents of the tubes are still warm, add 1 ml of 40% Rochelle salt solution. Cool and read the intensity of dark red colour at 510 nm. Run a series of standards using glucose (0–500µg) and plot a graph (Miller, G.L. 1972).

Calculation

Calculate the amount of reducing sugars present in the sample using the standard graph.

3.3.2. Phenol Sulphuric Acid Method for Total Carbohydrate

The phenol sulphuric acid method is to estimate total carbohydrates present in the sample. In hot acidic medium glucose is dehydrated to hydroxyl methyl furfural. This

forms a green colored product with phenol and has absorption maximum at 490 nm. 100mg of the cotton and algal sample was weighed and taken in a boiling tube. It is hydrolyzed by keeping it in a water bath for 3hours with 5ml of 2.5N HCL and cooled at room temperature. It is then neutralized with solid sodium carbonate until the effervescence ceases. It is then made up to 100ml and centrifuged. 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard is pipetted out into a series of test tubes. 0.1 and 0.2ml of the cotton and sample solution is pipetted out in separate test tubes. The volume in each tube is made up to 1ml with water. The blank is set with 1ml of water. 1ml of phenol solution is added to each tube. 5ml of 96% sulphuric acid is added to each tube and shake well. After 10 min the contents in the tubes are shaken and placed in the water bath at 25–30°C for 20 min. The colour is read at 490 nm. The amount of total carbohydrate present in the sample solution is calculated using the standard graph (Dubois.M *et al.* 1956)

Calculation

Absorbance corresponds to 0.1ml of the test = x mg of glucose

100ml of the sample solution contains =0.1x×100 mg of glucose

= % of total carbohydrate present.

3.3.3. Cellulose estimation

The glucose concentration was used to determine the cellulose concentration.

%w/w cellulose content = 0.9/0.96x C 1 x (V/M) x α x 100

0.9 is the coefficient that results from the molecular weight ratio of the polymer and the monomer hexose. The saccharification yield was taken as 0.96, C 1 as the glucose concentration (g/L), V the total volume of sugar solution (L), M the dry weight of the algal biomass sample (g) and α is the dilution of the sample (Miller *et al*., 1959).

3.3.4. Hemi cellulose estimation

The glucose concentration and the reducing sugar concentration were used to estimate the hemicelluloses content.

% w/w hemi cellulose content = 0.88/0.93 x C 2- C 1 x (V/M) x α x 100

0.88 is the coefficient that results from the molecular weight ratio of the polymer and the monomer pentose, 0.93 is the saccharification yield of xylane to xylose, C2 is the determined reducing sugars concentration (g/L) from the DNS method, C1 the glucose concentration (g/L) from above, V the total volume of sugar solution (L), M the dry weight of the algal biomass sample (g) and α the dilution of the sample (Miller *et al*., 1959).

3.3.5. Lignin and Ash content

The solid residue obtained after treatment of sample was dried at 105°C for 24 hours and weight (W1). The residue was then transferred to a pre-weighed dry porcelain crucible and heated at 600°C for 5 hours. After cooling down, it was weighed (W2) and ash content (%) was determined. Acid insoluble lignin was then calculated by the difference (W1-W2) (Ververis *et al*., 2004).

3.4. Optimization of algae

The effect of different parameters on the growth rate of algae was assessed by following completely randomized experimental design. In these experiments only one factor was variable while all other conditions were kept constant. The culture conditions which controlled for the algal growth were nutrients, light and pH (N. Munir *et al.,* 2015).

3.4.1. Culture medium/Nutrients

For monitoring the best growth of algae both the media (BB and BG) were used for culturing algal cells and the best growth rate was estimated after a required period of time has been passed. The calculation was made on 1^{st}, 3^{rd}, 5^{th}, 7^{th} day and the rate was estimated on the basis of the rate of increase in fresh weight of biomass. The cell mass was separated through filtration and then weighted after blotting the excess water.

3.4.2. pH

Both the culture media were adjusted with various pH ranges i.e., 4, 6, 7, 8 and 10. Algae samples were grown within these pH ranges and the effect was observed and measured by calculating the fresh weight of samples after the specified time period has been elapsed.

3.4.3. Light

Algal cultures were placed out door in open sunlight, inside the lab near window and under artificial light provided by fluorescent tubes. The effect of light on the growth of algae was calculated by measuring the algal biomass after 7 days.

3.5. Algal cultivation and optimization in tanks

The Algae were cultivated in water tank to study the algal growth. To 500L of water, 250g of glucose was taken in a water tank. 10g of algal sample was inoculated into the tank. The glucose was provided in the time interval of 5 days. After 10 days the algal biomass was taken and weighed. It is compared with normal algal growth in 500L of water without the presence of glucose.

3.6 Sample preparation

3.6.1. Paper pulp preparation

The paper pulp was obtained from paper mill- Gajendra paper and boards limited, Chinnakalaiyamuthur, palani Road, Udumalpet. The pulp was added with 1000ml distilled water and poured in a tub.

3.6.2. Algal pulp preparation

The collected algal sample was dried (shallow drying) and then it was cleared of all the debris material. Then the algal material was separated out as thin thread like forms and was grounded in blender. With this, humus, waste cotton and waste pulp were added and grounded in blender. The pulp was prepared in different ratios; 1:1:1:2, 1.5:1.5:1:1, 2:1:1:1, 3:2:1:1, 4:2:1:1 and 5:2:1:1. 150 ml of this pulp is added in 1000ml of distilled water. Then the pulp is poured into the tub and the mixture is mixed thoroughly. It is used for papermaking procedure.

3.7. Handmade paper making

3.7.1. Control paper

The paper pulp is poured into a tub and the process is carried out till the slurry is about 4 inches. To make thin paper, more water is needed. The lifting mould (a mesh on a wooden frame) is dipped into the tub and shaken evenly and lifted out with pulp on it and the water is drained. Shake gently to distribute the fibers freely. The consistency of the pulp in the tank should be kept constant all the time. Once the sheet is formed, the frame was

separated and mesh is placed upside down and transferred onto a soaking cloth placed on the plywood. The mesh is removed and another cloth is placed above the wet pulp. Then another ply wood is placed above this cloth and pressed for few minutes to remove the water from the pulp. The steps are repeated by changing the cloth until there is no more water to be soaked manually. Pressing reduces the thickness of the paper and the sheets become more compact. This process improves the physical properties of the paper and drying.

3.7.2. Algal pulp

The microalgae pulp that was prepared was added as supplements to paper pulp in the concentration of 10-50%. The paper pulp concentration was decreased respectively with respect to the increasing algae pulp concentration. Then the similar steps as of the control paper making were followed. The resulting paper quality was documented.

3.7.3. Drying

Even after the sheets have been pressed, they still contain about 50% to 65% of the moisture. The sheets are hung in the sunlight to dry. Solar drying can speed up the process and minimize the space required for drying.

3.8. Quality of paper:

Testing the quality of the paper by physical and optical properties: Basis weight, bursting strength, cobb factor air permeability, pore size and ply bond factor were checked. The paper quality was checked in paper mill-Gajendra paper and boards limited, Palani.

RESULTS AND DISCUSSION

4.1 Sample collection:

Algal samples both Microalgae and Macroalgae were collected and taken for further identification, characterization, biochemical analysis and then for pulp supplement. Microalgal samples were collected from fresh water bodies, Kanyakumari and effluent treatment plants and the macroalgae were collected from the marine areas.

4.2 Morphological Characterization:

The collected samples were identified and characterized based on their morphological/microscopic features.

4.2.1 Microalgae:

The wet mount technique was performed on the microalgal samples confirm that the algae found here belongs to the Cyanobacteria and Chlorophyta genus. Some of the members were found to be *Oscillatoria sp*., *Microcystis sp*., *Spirulina sp., Phormidium sp., Nostoc sp., Planktothrix sp., Trichodesmium sp.,* and *Microchaete sp*., *Diatoms.* The morphological features of the isolated microalgae were verified in the laboratory, compared with internet images and review papers, inorder to confirm the identification.The characteristics of the identified microalgae were then referred in standard algogy manuals and have been discussed below.The morphological identification of microalgal samples have been shown in plate 1.

Table- 1: Collection of Microalgae samples from varies places

S.NO.	LOCATION OF SAMPLE	MICROALGAE
1	Fresh water	*Osillatoria sp.,*
2	Fresh water	*Planktothrix sp.,*
3	Effulent treatment plant	*Trichodesmium sp.,*
4	Fresh water	*Osillatoria sp.,*
5	Fresh water	*Osillatoria sp.,*

6	Marine areas	*Spirulina sp.,*
7	Fresh water	*Osillatoria sp.,*
8	Effulent treatment plant	*Phormidium sp.,*
9	Fresh water	*Osillatoria sp.,*
10	Fresh water	*Osillatoria sp.,*
11	Fresh water	*Osillatoria sp.,*
12	Fresh water	*Osillatoria brevis*
13	Effulent treatment plant	*Microcystis sp.,*
14	Fresh water	*Osillatoria sp.,*
15	Effulent treatment plant	*Phormidium sp.,*
16	Marine areas	*Nostoc sp.,*
17	Fresh water	*Microcystis sp.,*
18	Marine areas	*Microchaete sp.,*
19	Marine areas	*Diatom*
20	Fresh water	*Nostoc sp.,*

4.2.2

Macroalgae:

The five macroalgae samples were chosen for analysis consists of two each of red, brown and two of green algae. These are (*Gracilaria sp., Sargassum sp., Chaetomorpha sp., Ulva sp., Kappaphycus sp.,*). The morphological features of the isolated macroalgae were again verified in the laboratory, compared with internet images and review papers, inorder to confirm the identification. The morphological identification of macroalgal samples have been shown in a plate 2.

Table- 2: Collection of Macroalgae samples from varies places

S.NO.	LOCATION OF SAMPLE	MACROALGAE
1	Marine areas	*Gracilaria sp.,*
2	Marine areas	*Ulva sp.,*
3	Marine areas	*Sargassum sp.,*

4	Marine areas	*Chaetomorpha sp.,*
5	Marine areas	*Kappaphycus sp.,*

4.3 Cultural characteristics:

The first algal growth was observed after a period of days and the active growth was observed for in the next 10 days. During the first 20 days the medium became light green in colour from colorless and for the next 10 days it became rapidly dark green showing intense algal growth in this period.

4.3.1 Morphological characterization:

The cultured microalgae samples showed similar microscopic morphology that was observed during the first morphological characterization. Thus the algal samples that were collected were cultivated successfully under the laboratory conditions and the morphology of these cultures was observed once again. The morphological features were compared with review articles. (Fritsch Classification). Plate 1 & 2. The observed samples were compared and reconfirmed with internet images and review papers.

4.5 Biochemical characterization of Micro and Macroalgae:

4.5.1 Estimation of Reducing sugar: (Miller *et al.,)*

The reducing sugar values were determined by using the standard graph formulae given in the Miller *et al.,* method.

Reducing sugar concentration (mg/ml) = 0.4534/A570 *nm*

Table- 3: Reducing sugar concentration of the ten microalgae samples in percentage

S. No.	Sample	Reducing sugar (%)

1	*Osillatoria sp.,*	0.9253
2	*Planktothrix sp.,*	0.7817
3	*Trichodesmium sp.,*	1.079
4	*Osillatoria sp.,*	0.7556
5	*Osillatoria sp.,*	0.855
6	*Spirulina sp.,*	1.007
7	*Osillatoria sp.,*	0.9646
8	*Phormidium sp.,*	0.8554
9	*Osillatoria sp.,*	1.135
10	*Osillatoria sp.,*	0.440

Fig- 3: Reducing sugar concentration of ten microalgae samples in percentage

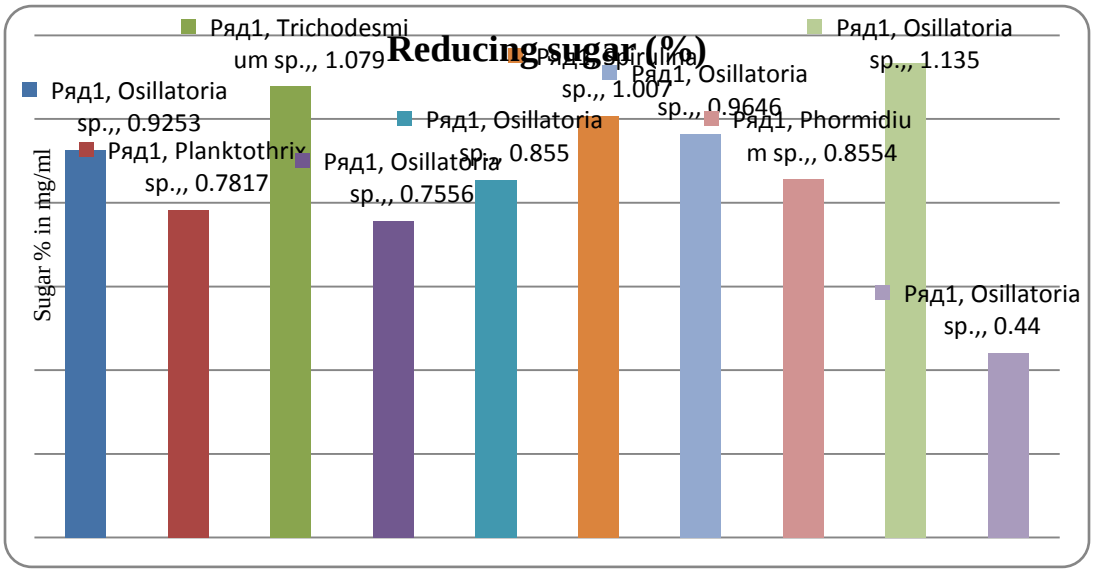
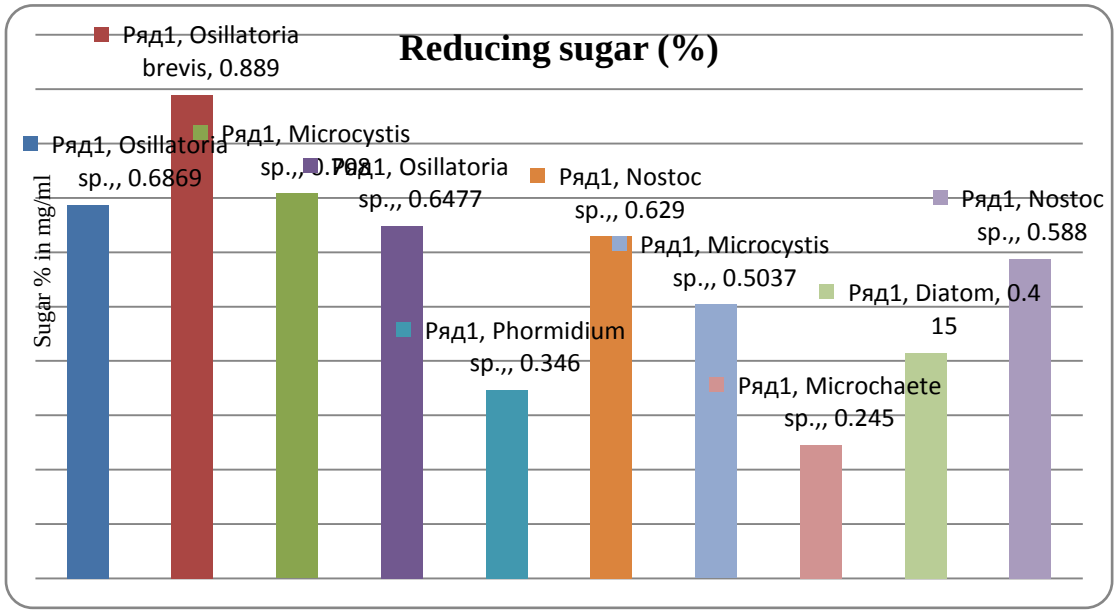


Table- 4: Reducing sugar concentration of the next ten microalgae samples in percentage

S.No.	Sample	Reducing sugar (%)
1	*Osillatoria sp.,*	0.6869

2	*Osillatoria brevis*	0.889
3	*Microcystis sp.*,	0.708
4	*Osillatoria sp.*,	0.6477
5	*Phormidium sp.*,	0.346
6	*Nostoc sp.*,	0.629
7	*Microcystis sp.*,	0.5037
8	*Microchaete sp.*,	0.245
9	*Diatom*	0.415
10	*Nostoc sp.*,	0.588

Fig- 4: Reducing sugar concentration of next ten microalgae samples in percentage

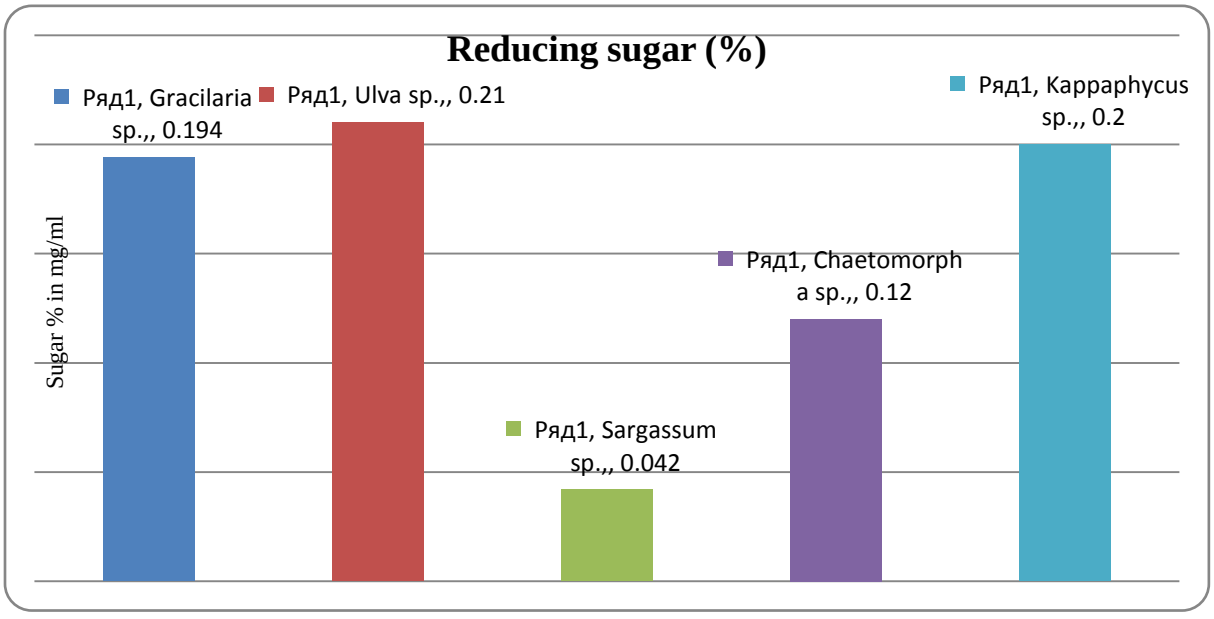


The highest level of reducing sugar present in the microalgal samples is 1.135% in *Osillatoria sp.*, followed by *Trichodesmium sp.*,showed (1.079%), *Spirulina sp.*, showed (1.007%), *Osillatoria sp.*,and the lowest being that of *Microchaete sp.*,(0.234%)

Table- 5: Reducing sugar concentration of the Macroalgae samples in percentage

S. No.	Sample	Reducing sugar (%)
1	*Gracilaria sp.,*	0.194
2	*Ulva sp.,*	0.21
3	*Sargassum sp.,*	0.042
4	*Chaetomorpha sp.,*	0.12
5	*Kappaphycus sp.,*	0.20

Fig- 5: Reducing sugar concentration of Macroalgae samples in percentage



The highest level of reducing sugar present in the macroalgae samples is 0.21% in *Ulva sp.,* followed by *Kappaphycus sp.,* showed (0.20%), followed by *Gracilaria sp.,* showed (0.194), followed by *Chaetomorpha sp.,* showed (0.12) and the lowest being that of *Sargassum sp.,* (0.042%).

4.5.2 Estimation of Glucose: (Anthrone method)

The glucose levels in the samples were determined after plotting the standard graph.

Table -6: Standard Glucose concentration and OD values

Concentration of Glucose(µg)	Optical density (nm)
20	0.12
40	0.26
60	0.40
80	0.54
100	0.67

Fig- 6: Standard graph of Glucose concentration

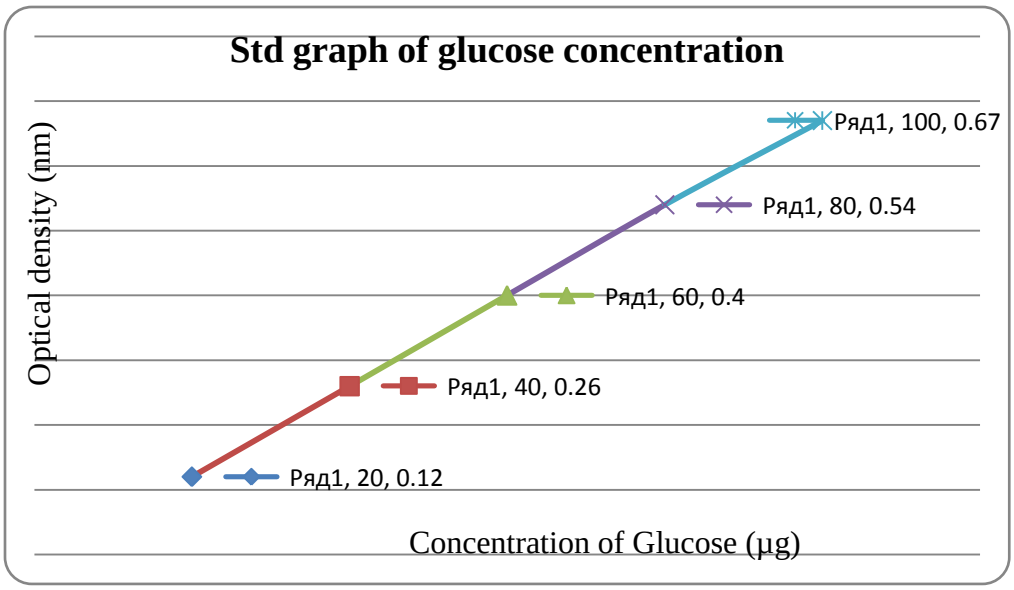


Table -7: Glucose concentration of ten microalgae samples in percentage

S. No.	Sample	Glucose (%)
1	*Osillatoria sp.,*	0.064
2	*Planktothrix sp.,*	0.045
3	*Trichodesmium sp.,*	0.035
4	*Osillatoria sp.,*	0.076
5	*Osillatoria sp.,*	0.056
6	*Spirulina sp.,*	0.023
7	*Osillatoria sp.,*	0.063
8	*Phormidium sp.,*	0.012
9	*Osillatoria sp.,*	0.067
10	*Osillatoria sp.,*	0.041

Fig-7: Glucose concentration of ten microalgae samples in percentage

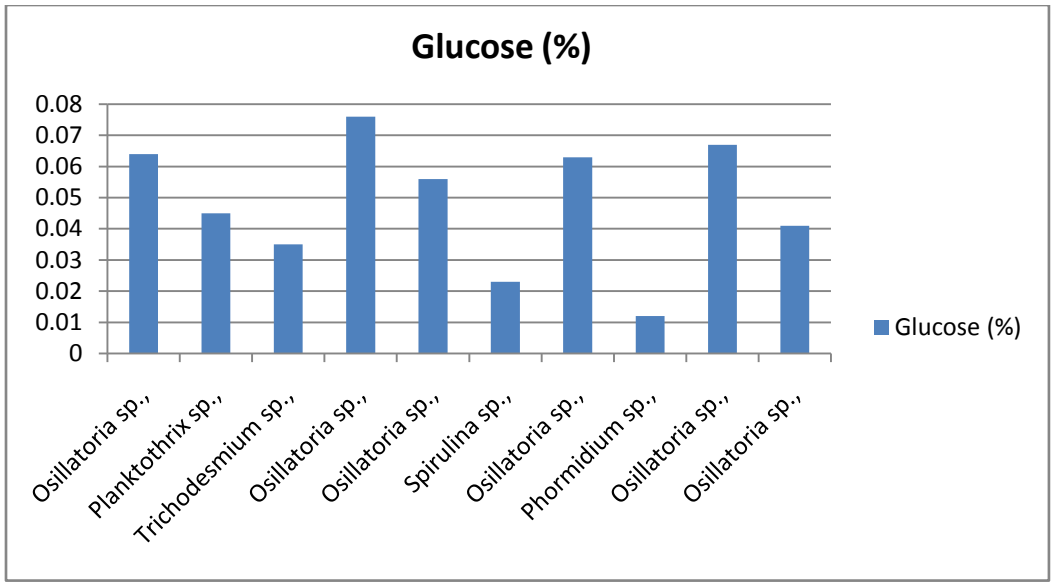
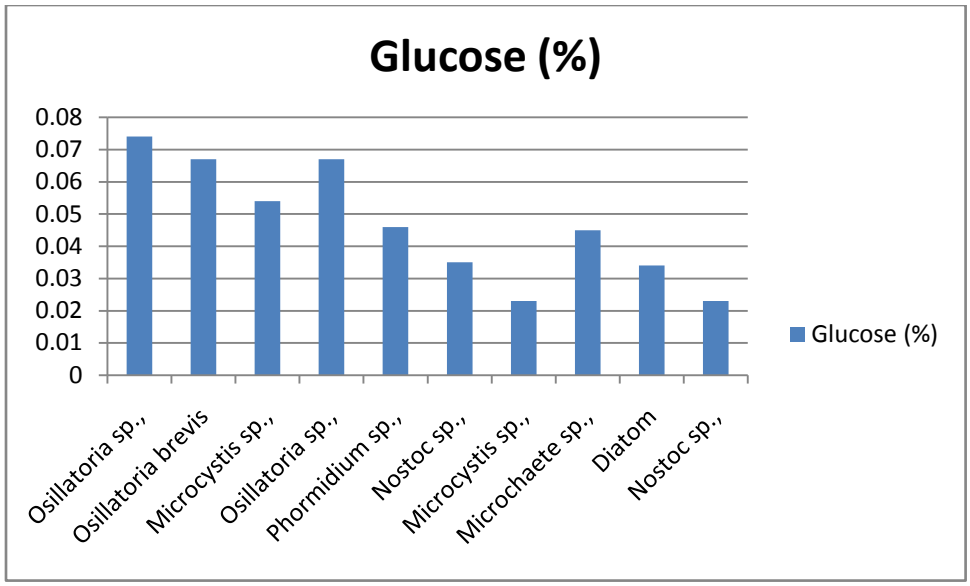


Table -8 Glucose concentration of next ten microalgae samples in percentage

S.No.	Sample	Glucose (%)
1	*Osillatoria sp.,*	0.074
2	*Osillatoria brevis*	0.067
3	*Microcystis sp.,*	0.054
4	*Osillatoria sp.,*	0.067
5	*Phormidium sp.,*	0.046
6	*Nostoc sp.,*	0.035
7	*Microcystis sp.,*	0.023
8	*Microchaete sp.,*	0.045
9	*Diatom*	0.034
10	*Nostoc sp.,*	0.023

Fig -8 Glucose concentration of next ten microalgae samples in percentage

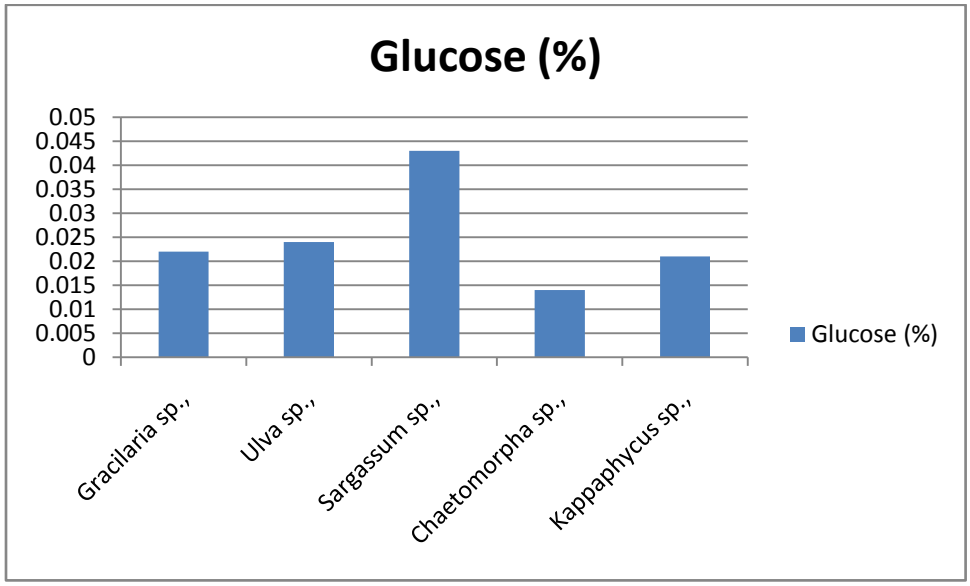


The glucose values showed 0.076 % in *Osillatoria sp.*, followed by *Microcystis sp.*,(0.054%) and another species of *Microcystis* showed (0.023%) and lowest being that of 0.012% *Phormidium sp.,*

Table -9 Glucose concentration of macroalgae samples in percentage

S. No.	Sample	Glucose (%)
1	*Gracilaria sp.,*	0.022
2	*Ulva sp.,*	0.024
3	*Sargassum sp.,*	0.043
4	*Chaetomorpha sp.,*	0.014
5	*Kappaphycus sp.,*	0.021

Fig -9 Glucose concentration of macroalgae samples in percentage



The highest glucose values showed 0.043% in *Sargassum sp.,* followed by *Ulva sp., Gracilaria sp., Kappaphycus sp.,* and lowest being that of *Chaetomorpha sp.,*(0.014%)

4.5.3 Estimation of Cellulose: (Miller *et al.,* 1959)

The cellulose content of the samples was determined by substituting the glucose concentration formulae given in the Miller *et al.,* 1959.

Table -10: Concentration of Cellulose in percentage for ten microalgae samples

S. No.	Sample	Cellulose (%)
1	*Osillatoria sp.,*	0.124
2	*Planktothrix sp.,*	0.147
3	*Trichodesmium sp.,*	0.101
4	*Osillatoria sp.,*	0.131
5	*Osillatoria sp.,*	0.138
6	*Spirulina sp.,*	0.184
7	*Osillatoria sp.,*	0.234
8	*Phormidium sp.,*	0.201

9	*Osillatoria sp.*,	0.173
10	*Osillatoria sp.*,	0.238

Fig -10: concentration of Cellulose in percentage for ten microalgae samples

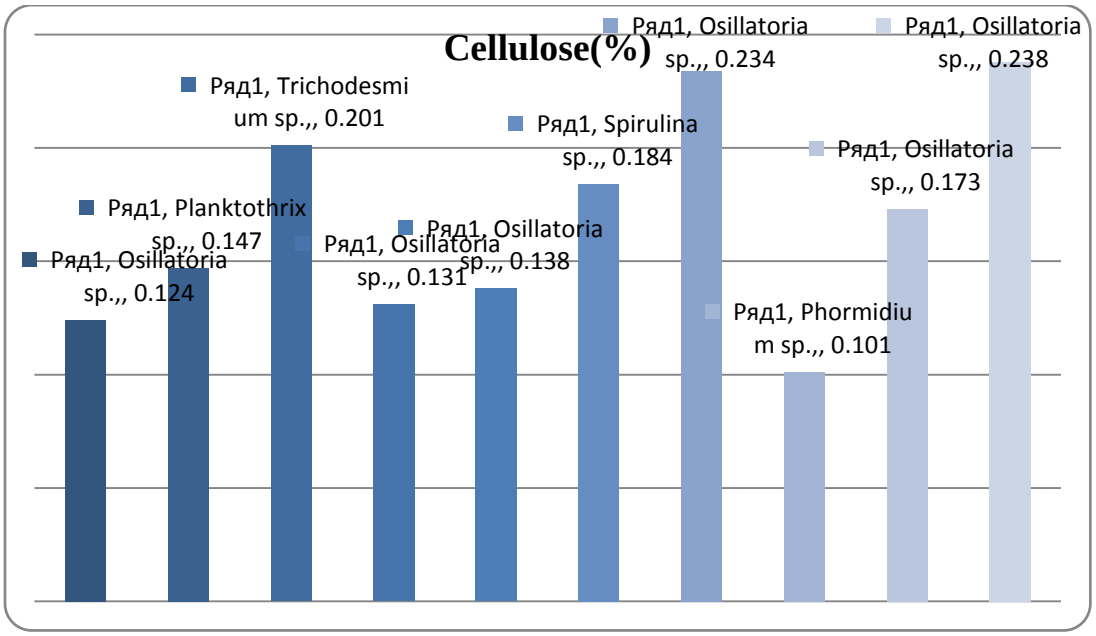
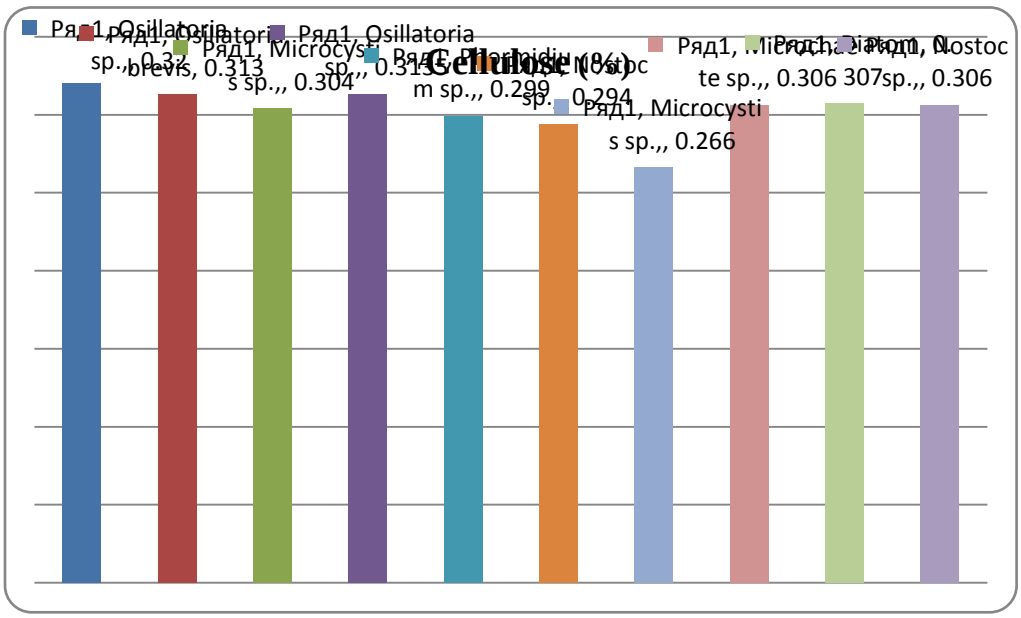


Table -11: Concentration of Cellulose in percentage for next ten microalgae samples

S. No.	Sample	Cellulose (%)
1	*Osillatoria sp.*,	0.320
2	*Osillatoria brevis*	0.313
3	*Microcystis sp.*,	0.304
4	*Osillatoria sp.*,	0.3135
5	*Phormidium sp.*,	0.299
6	*Nostoc sp.*,	0.294
7	*Microcystis sp.*,	0.266
8	*Microchaete sp.*,	0.306

9	*Diatom*	0.307
10	*Nostoc sp.*,	0.306

Fig -11: Concentration of Cellulose in percentage for next ten microalgae samples



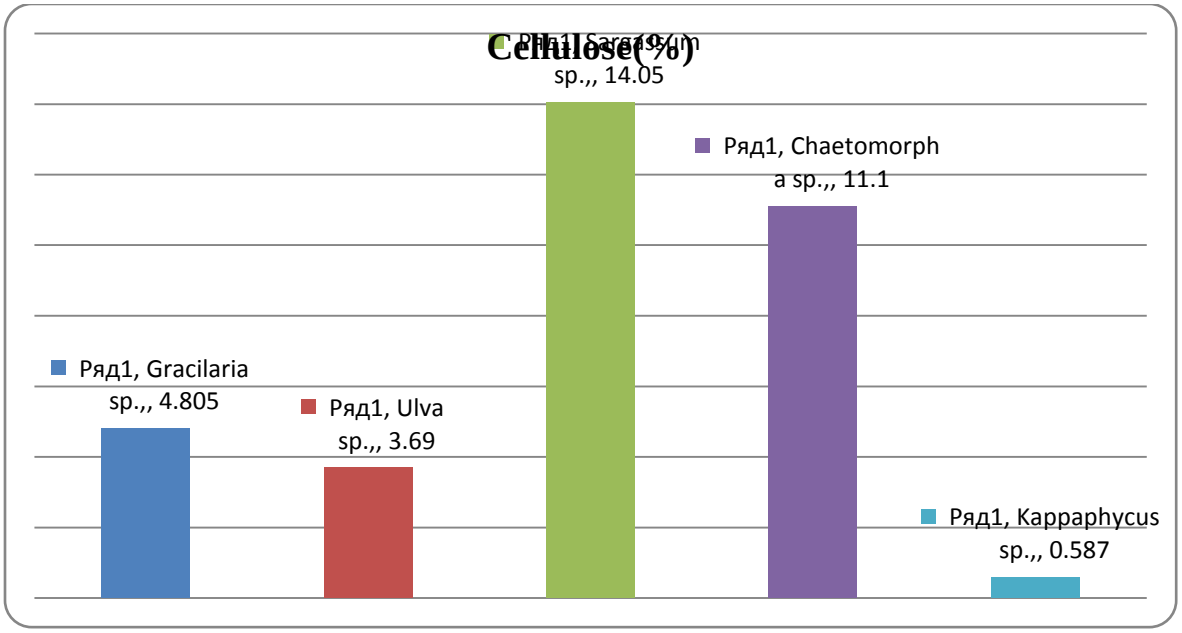
The highest level of Cellulose present in the microalgal samplesis 0.320% in *Osillatoria sp.*, and the lowest being that of *Trichodesmium sp.*,(0.101%). The higher the amount of cellulose present in the sample more suitable it is for the production of paper. Thus the higher cellulose content organisms could be studied and experimented more for the production of paper.

Table -12: Concentration of Cellulose in percentage for macroalgae samples

S. No.	Sample	Cellulose (%)

1	*Gracilaria sp.,*	4.805
2	*Ulva sp.,*	3.69
3	*Sargassum sp.,*	14.05
4	*Chaetomorpha sp.,*	11.1
5	*Kappaphycus sp.,*	0.587

Fig -12: Concentration of Cellulose in percentage for macroalgae samples



The highest level of Cellulose present in the macroalgae samples is 14.05% in *Sargassum sp.*,followed by *Chaetomorpha sp., Gracilaria sp., Ulva sp.*, and the lowest being that of *Kappaphycus sp.*,(0.587%).

4.5.4 Estimation of Hemicellulose: (Ververis *et al.*, 2004)

The hemicelluloses content of the samples was determined by substituting the reducing sugar and glucose in the formulae by Ververis *et al., 2004.*

Table- 13: Concentration of Hemicellulose in percentage for ten microalgae samples

S. No.	Sample	Hemicellulose (%)
1	*Osillatoria sp.,*	0.662
2	*Planktothrix sp.,*	0.234
3	*Trichodesmium sp.,*	0.435
4	*Osillatoria sp.,*	0.766
5	*Osillatoria sp.,*	0.704
6	*Spirulina sp.,*	0.529
7	*Osillatoria sp.,*	0.484
8	*Phormidium sp.,*	0.620
9	*Osillatoria sp.,*	0.446
10	*Osillatoria sp.,*	1.376

Fig- 13: Concentration of Hemicellulose in percentage for ten microalgae samples

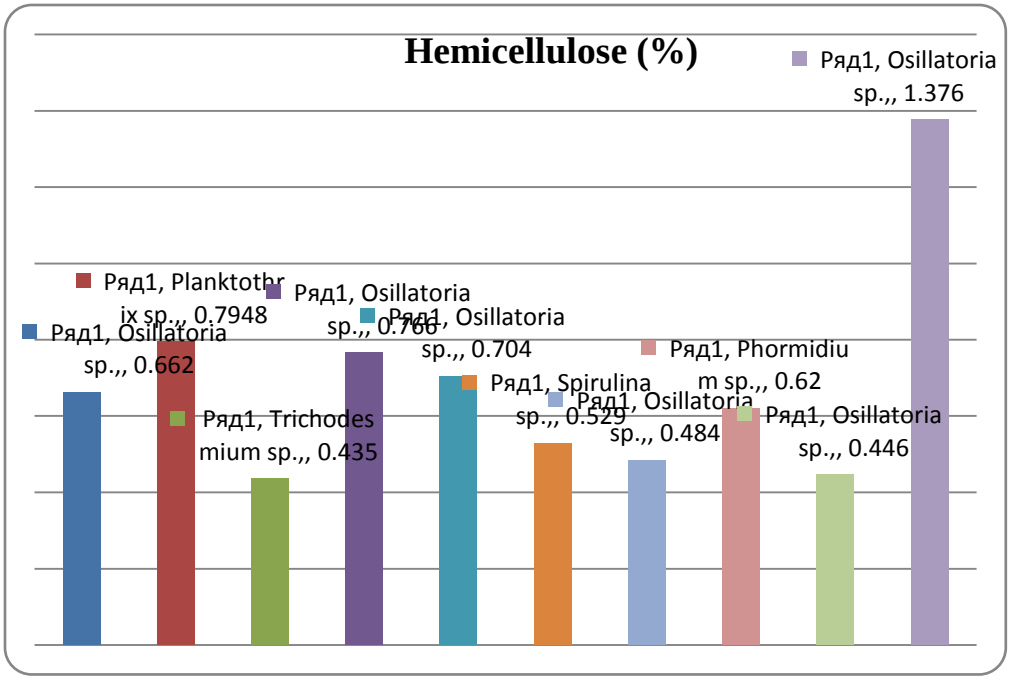
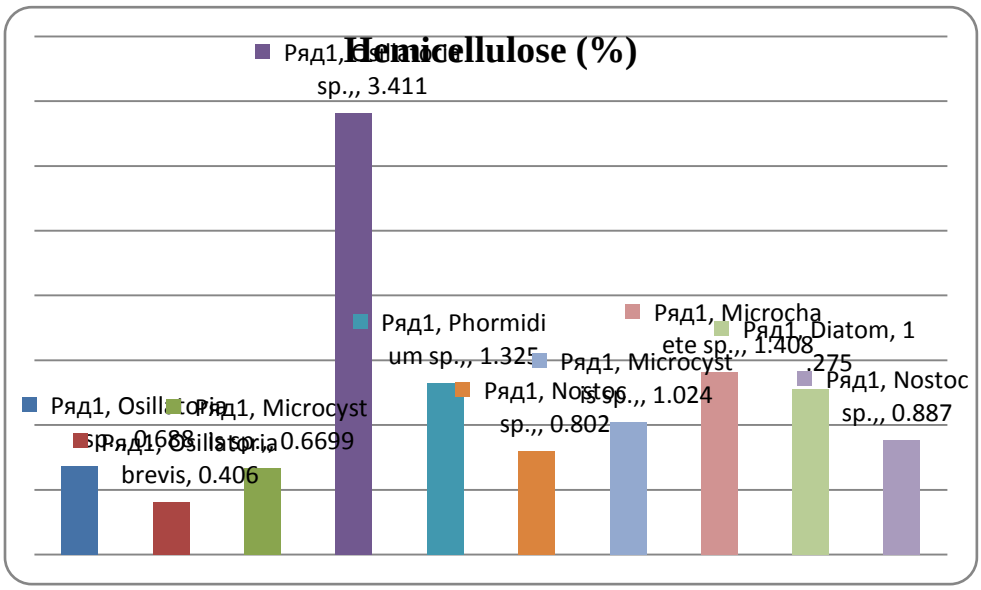


Table- 14: Concentration of Hemicellulose in percentage for next ten microalgae samples

S. No.	Sample	Hemicellulose (%)
1	*Osillatoria sp.,*	0.688
2	*Osillatoria brevis*	0.406
3	*Microcystis sp.,*	0.6699
4	*Osillatoria sp.,*	3.411
5	*Phormidium sp.,*	1.325
6	*Nostoc sp.,*	0.802
7	*Microcystis sp.,*	1.024
8	*Microchaete sp.,*	1.408
9	*Diatom*	1.275
10	*Nostoc sp.,*	0.8870

Fig- 14: Concentration of Hemicellulose in percentage for next ten microalgae samples

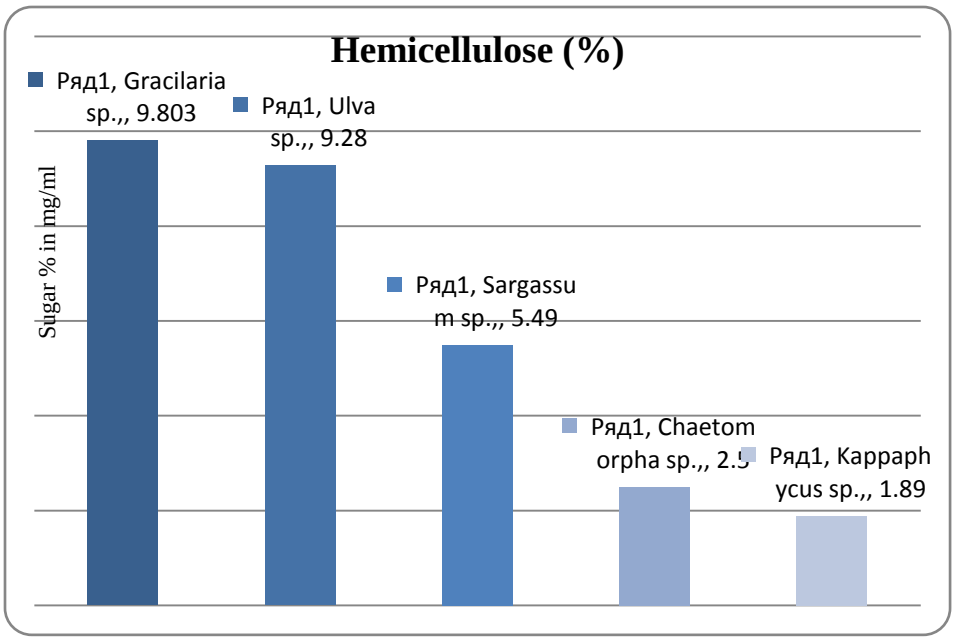


The highest level of hemicellulose present in the microalgal samples is 3.411% in *Osillatoria sp.*, followed by *Osillatoria sp.*, (1.376%), *Microchaete sp.*, (1.408%), *Diatom* (1.275%) and the lowest being that of *Planktothrix sp.*, 0.234%.

Table- 15: Concentration of Hemicellulose in percentage for macroalgae samples

S. No.	Sample	Hemicellulose (%)
1	*Gracilaria sp.,*	9.803
2	*Ulva sp.,*	9.28
3	*Sargassum sp.,*	5.49
4	*Chaetomorpha sp.,*	2.5
5	*Kappaphycus sp.,*	1.89

Fig- 15: Concentration of Hemicellulose in percentage for macroalgae samples



The highest level of hemicellulose present in the macroalgae samples is 9.803% in *Gracilaria sp.*, and the lowest being that of *Kappaphycus sp.*,(1.89%). Hemicelluloses content also plays an important role in paper making, the more the value of it, better suitability for paper making.

The total carbohydrate content the micoralgae range from 8 % in *Spirulina platensis* to a maximum of 64% in *Microcystis sp.,*. reported in Indira Priyadarshani *et al.*, and seaweeds have carbohydrate concentration ranging from 16.9 % in *Ulva reticulata* to 49.5 % in *Caulerpa sp.*, reported in N. Kaliaperumal *et al.*, 1995. The values of carbohydrates estimated in the present study are comparatively less regarding the microalgae and could be same as the lower range of seaweeds.

4.5.5 Estimation of Lignin: (Ververis *et al.*, 2004)

The lignin content of the samples was determined by Ververis *et al.*, *2004.*

Table-16: Concentration of Lignin in percentage for ten microalgae samples

S. No.	Sample	Lignin (%)
1	*Osillatoria sp.,*	0.535

2	*Planktothrix sp.,*	0.574
3	*Trichodesmium sp.,*	0.13
4	*Osillatoria sp.,*	0.462
5	*Osillatoria sp.,*	0.279
6	*Spirulina sp.,*	0.815
7	*Osillatoria sp.,*	0.236
8	*Phormidium sp.,*	0.3653
9	*Osillatoria sp.,*	0.5763
10	*Osillatoria sp.,*	0.714

Fig- 16: Concentration of Lignin in percentage for ten microalgae samples

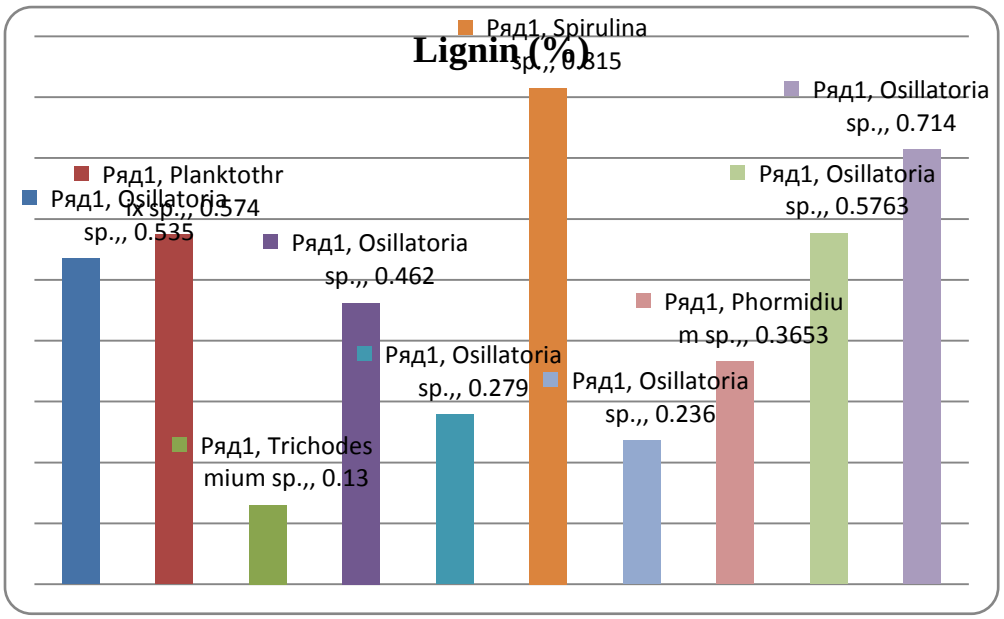
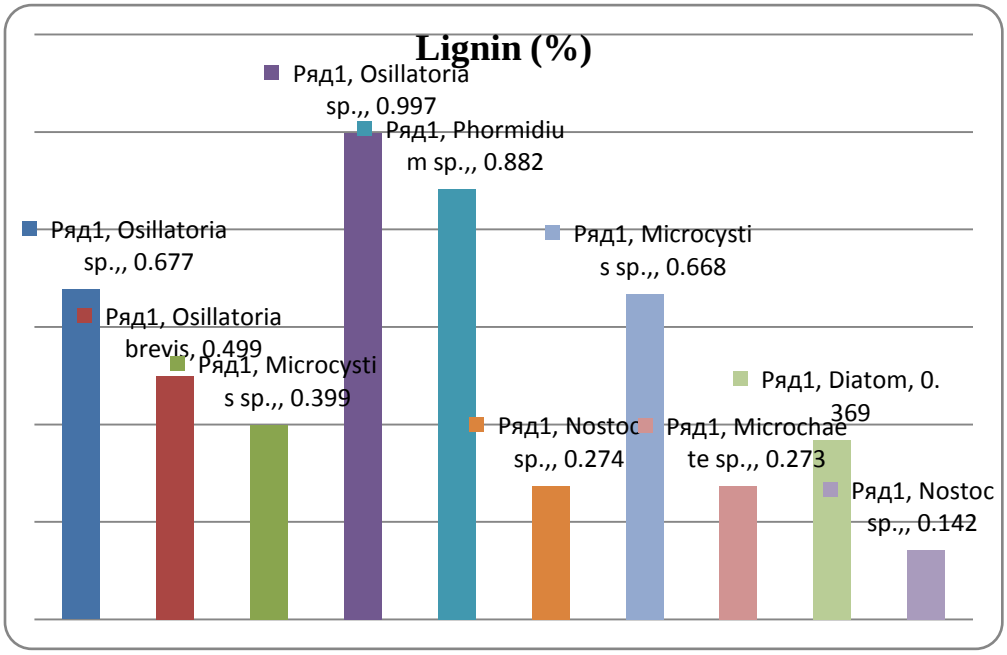


Table-17: Concentration of Lignin in percentage for next ten microalgae samples

S. No.	Sample	Lignin (%)
1	*Osillatoria sp.,*	0.677
2	*Osillatoria brevis*	0.499
3	*Microcystis sp.,*	0.399
4	*Osillatoria sp.,*	0.997
5	*Phormidium sp.,*	0.882
6	*Nostoc sp.,*	0.274
7	*Microcystis sp.,*	0.668
8	*Microchaete sp.,*	0.273
9	*Diatom*	0.369
10	*Nostoc sp.,*	0.142

Fig- 17: Concentration of Lignin in percentage for next ten microalgae samples

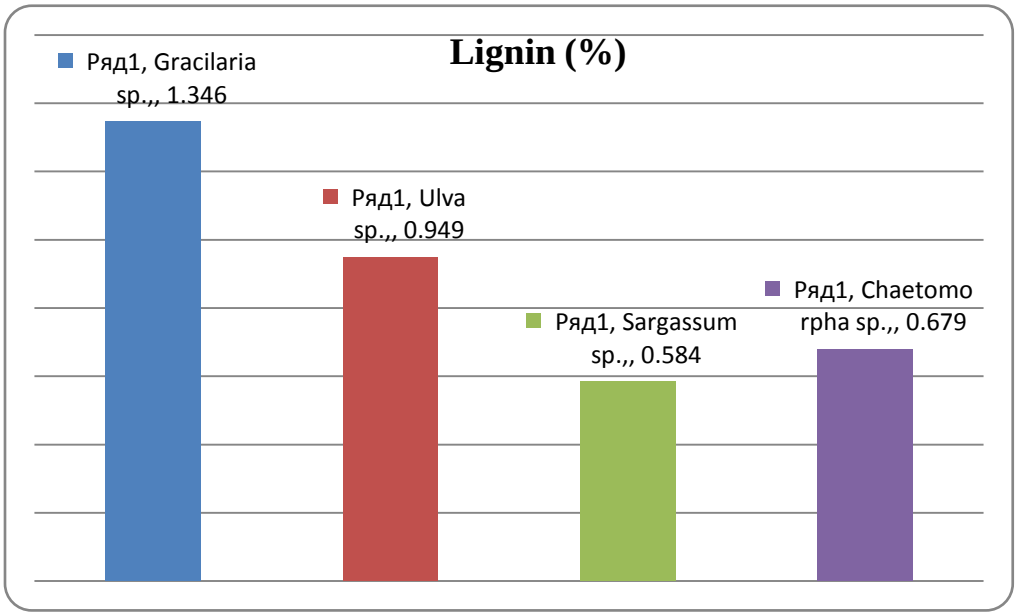


The highest level of lignin present in the microalgae samples is 0.997% in *Osillatoria sp.*, and the lowest being that of *Trichodesmium sp.*,(0.13%).

Table-18: Concentration of Lignin in percentage for macroalgae samples

S. No.	Sample	Lignin (%)
1	*Gracilaria sp.,*	1.346
2	*Ulva sp.,*	0.949
3	*Sargassum sp.,*	0.584
4	*Chaetomorpha sp.,*	0.679

Fig- 18: Concentration of Lignin in percentage for macroalgae samples



The highest level of lignin present in the macroalgae samples is 1.346% in*Gracilaria sp.,* and the lowest being that of *Sargassum sp.,* (0.584%). The lignin content of the microalgae was 0.584% and 1.346% which was very less compared to the values reported by Velveris *et al.,* and as for seaweeds it has been reported in various articles to be nil or very extremely low. The lignin concentration plays a detrimental role in paper

making and hence as all the samples showed very low concentration of them they could act as a suitable supplement.

4.5.6 Estimation of Ash content:

Table-19: Concentration of Ash content in percentage for ten microalgae samples

S. No.	Sample	Ash (%)
1	*Osillatoria sp.,*	0.965
2	*Planktothrix sp.,*	0.321
3	*Trichodesmium sp.,*	0.481
4	*Osillatoria sp.,*	0.413
5	*Osillatoria sp.,*	0.310
6	*Spirulina sp.,*	0.510
7	*Osillatoria sp.,*	0.3281
8	*Phormidium sp.,*	0.214
9	*Osillatoria sp.,*	0.2687
10	*Osillatoria sp.,*	0.312

Fig- 19: Concentration of Ash content in percentage for ten microalgae samples

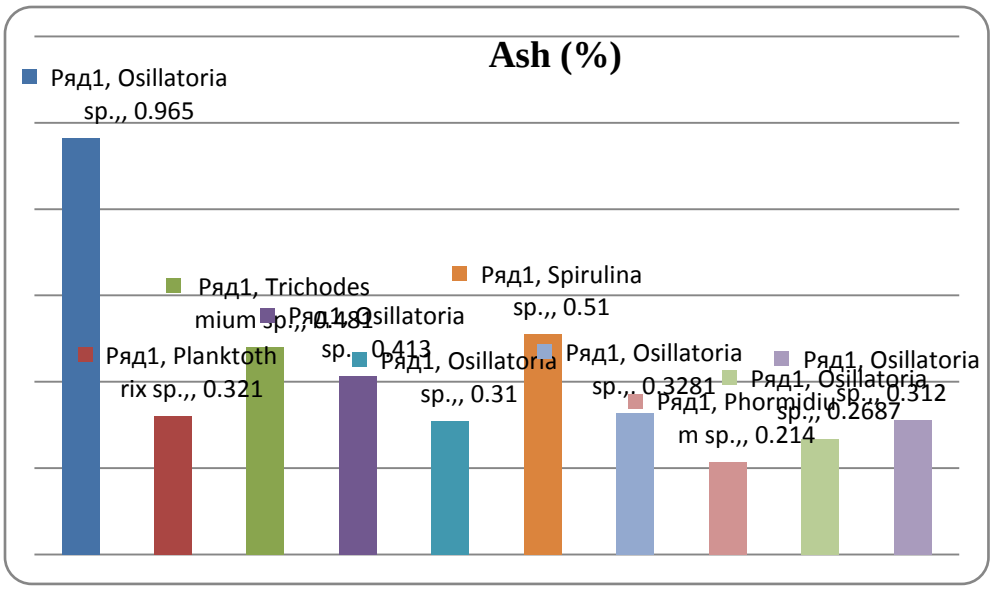
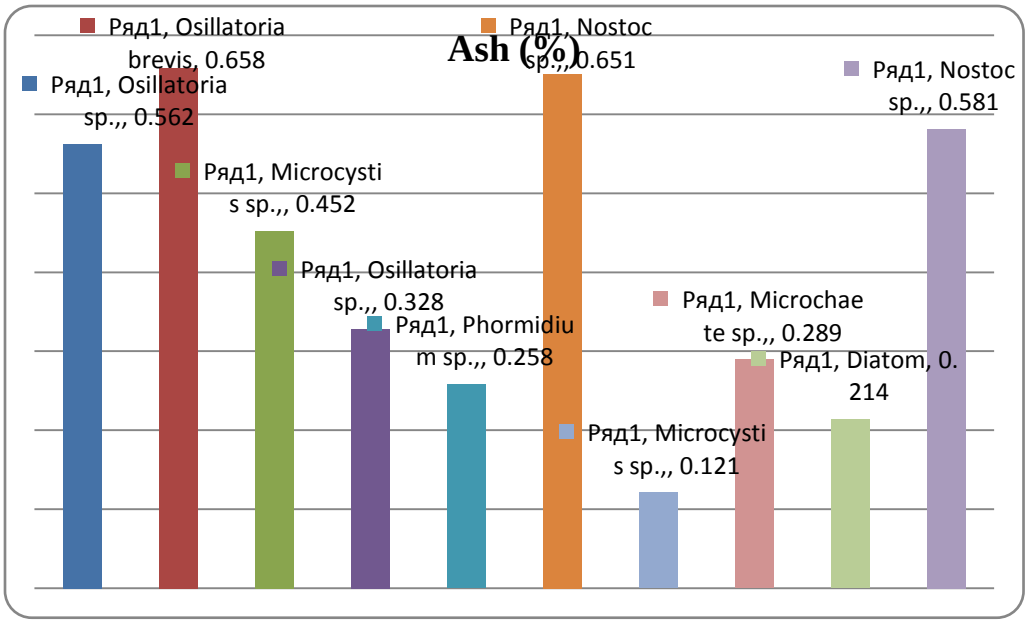


Table-20: Concentration of Ash content in percentage for next ten microalgae samples

S. No.	Sample	Ash (%)
1	*Osillatoria sp.,*	0.562
2	*Osillatoria brevis*	0.658
3	*Microcystis sp.,*	0.452
4	*Osillatoria sp.,*	0.328
5	*Phormidium sp.,*	0.258
6	*Nostoc sp.,*	0.651
7	*Microcystis sp.,*	0.121
8	*Microchaete sp.,*	0.289
9	*Diatom*	0.214
10	*Nostoc sp.,*	0.581

Fig- 20: Concentration of Ash content in percentage for next ten microalgae samples

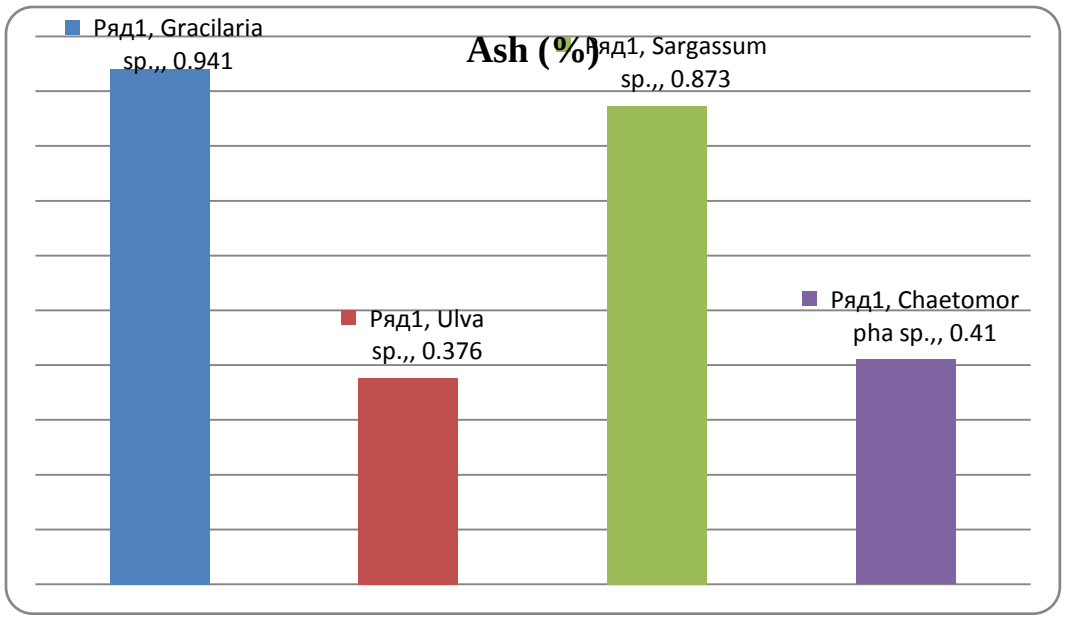


The highest level of ash content present in the microalgae samples is 0.965% in *Osillatoria sp.*, followed by another species *Osillatoria* showed (0.658%) followed by *Nostoc sp.*,(0.651%) and the lowest being that of *Microcystis sp.*,(0.121%).

Table-21: Concentration of Ash content in percentage for macroalgae samples

S. No.	Sample	Ash (%)
1	*Gracilaria sp.*,	0.941
2	*Ulva sp.*,	0.376
3	*Sargassum sp.*,	0.873
4	*Chaetomorpha sp.*,	0.410

Fig- 21: Concentration of Ash content in percentage for macroalgae samples



The highest level of ash content present in the macroalgae samples is 0.941% in *Gracilaria sp.*, and the lowest being that of *Ulva sp.*, (0.376%).

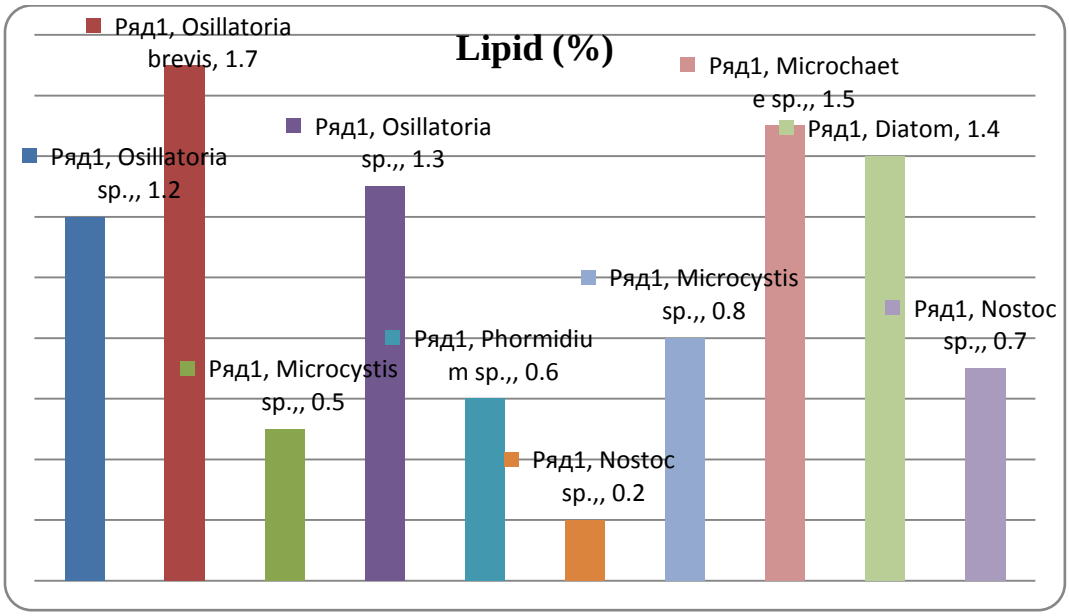
4.5.7 Estimation of Lipid: (EG Bligh, WJ Dyer., 1959)

The lignin content of the samples was determined by EG Bligh, WJ Dyer (1959)

Table-22: Concentration of Lipid in percentage for microalgae samples

S. No.	Sample	Lipid (%)
1	*Osillatoria sp.,*	1.2
2	*Osillatoria brevis*	1.7
3	*Microcystis sp.,*	0.5
4	*Osillatoria sp.,*	1.3
5	*Phormidium sp.,*	0.6
6	*Nostoc sp.,*	0.2
7	*Microcystis sp.,*	0.8
8	*Microchaete sp.,*	1.5
9	*Diatom*	1.4
10	*Nostoc sp.,*	0.7

Fig- 22: Concentration of Lipid in percentage for microalgae samples

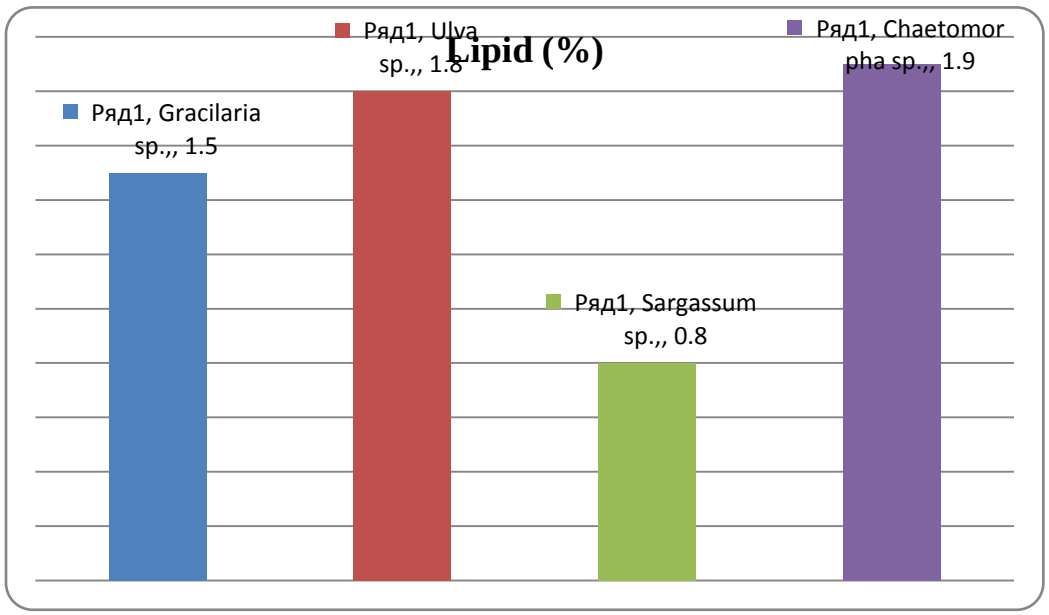


The highest level of lipid present in the microalgae samples is 1.7% in *Osillatoria brevis* and the lowest being that of *Nostoc sp.*, (0.2%).

Table-23: Concentration of Lipid in percentage for macroalgae samples

S. No.	Sample	Lipid (%)
1	*Gracilaria sp.*,	1.8
2	*Ulva sp.*,	1.9
3	*Sargassum sp.*,	1.5
4	*Chaetomorpha sp.*,	0.8

Fig- 23: Concentration of Lipid in percentage for macroalgae samples



The highest level of lipid present in the macroalgae samples is 1.9% in*Ulva sp.*,and the lowest being that of *Chaetomorpha sp.*, (0.8%

4.6 Pigment extraction: (Dere *et.al*., (1998) and Yoshii *et.al*., (2004)

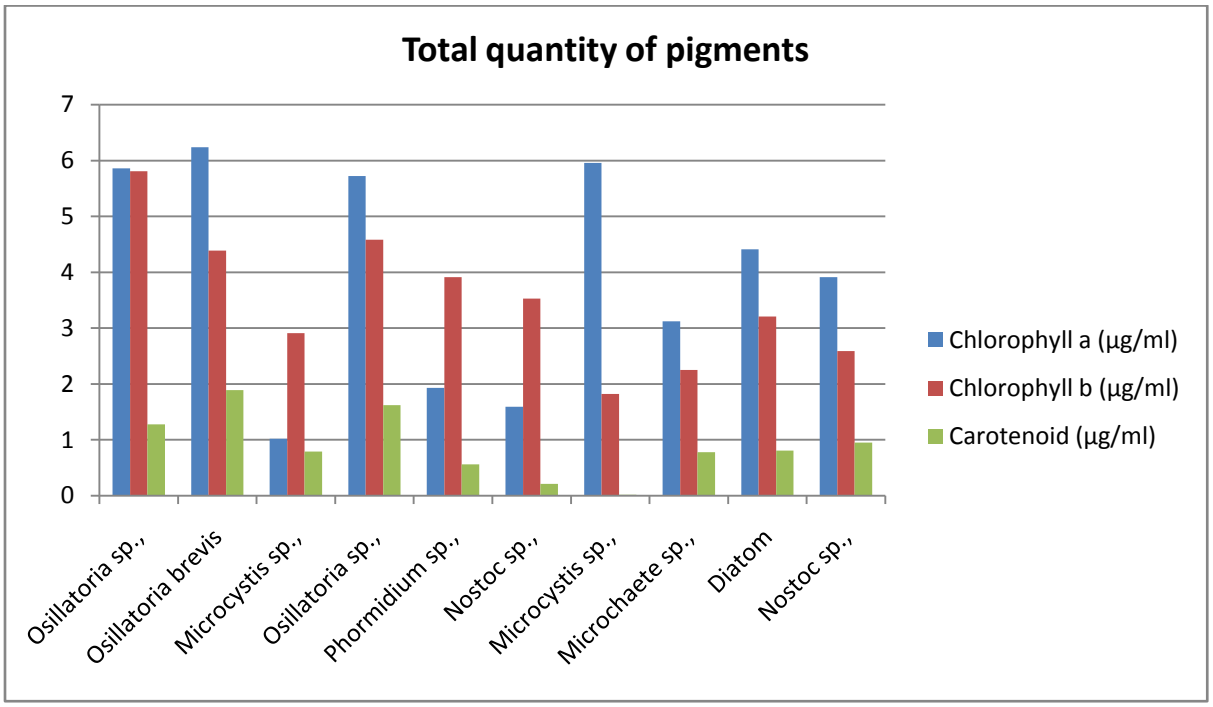
The pigment extraction of the samples was determined by Dere *et.al*., (1998) and Yoshii *et.al*., (2004) method.

Table-24: Total quantity of Pigments for microalgae samples

S.No.	Sample	Chlorophyll a (µg/ml)	Chlorophyll b (µg/ml)	Carotenoid (µg/ml)
1	*Osillatoria sp.*,	5.86	5.81	1.28
2	*Osillatoria brevis*	6.24	4.39	1.89
3	*Microcystis sp.*,	1.02	2.91	0.79
4	*Osillatoria sp.*,	5.72	4.58	1.62

5	*Phormidium sp.,*	1.93	3.91	0.56
6	*Nostoc sp.,*	1.59	3.53	0.21
7	*Microcystis sp.,*	5.96	1.82	0.02
8	*Microchaete sp.,*	3.12	2.25	0.78
9	*Diatom*	4.41	3.21	0.81
10	*Nostoc sp.,*	3.91	2.59	0.95

Fig- 24: Total quantity of Pigments for microalgae samples



The solvent extraction using acetone showed high amount of chlorophyll a (6.24µg/ml) in *Osillatoria brevis* and chlorophyll b (5.82µg/ml) in *Osillatoria sp.,* The solvent extraction using diethyl ether showed high amount of total carotene (1.89µg/ml) *Osillatoria brevis* in and low amount of total carotene (0.02µg/ml) in *Microcystis sp.,*

4.7 Application of Algae in Paper pulp supplement:

The use of algae as a supplement in ETP pulp was carried out successfully for microalgae samples showing positive results to an extent. This could be due to the presence

of substances like cellulose and hemicelluloses just like that of the plant pulp and higher amount in some cases. These are depicted in Plate 5.

4.7.1 Microalgae:

Table-25: Concentration of ETP pulp + algae concentration in paper making

Pulp concentration (%)	100	90	80	70	60	50
Microalgae concentration (%0	0	10	20	30	40	50

The handmade papers were made from10-50% algae concentration successfully (Plate 5). The quality of algal paper were tested in SPB mill and it was resulted in the below table.

Table-26: Parameter report of the algal paper

S.No.	Parameter	Unit	Control	28g ETP pulp+12g algae content	26g ETP pulp+14g algae content	24g ETP pulp+16g algae content	22g ETP pulp+18g algae content	20g ETP pulp+20g algae content
1	Gsm		79	76	76	78	82	80
2	Caliper	Micron	144	160	164	185	196	190
3	Bulk	Cc/g	1.82	2.1	2.1	2.3	2.40	2.40
4	Ash content	%	42.5	46.8	48.2	48.6	47.3	55.3
5	Tear Factor	m	35	13.0	15	11.0	8.6	7.5
6	Breaking Length	m	1100	480	516	390	350	280
7	Burst Factor	-	15.3	3.3	3.0	2.9	2.6	2.2
8	Brightness	%	32.0	25.2	25.8	25.5	24.1	23.8

The above results indicate that addition of algal content in paper pulp will not give sufficient strength properties for using wrapping purpose. However, small percentage of algal content addition with paper pulp may be used for egg tray production.

Second Year Project Results

Fresh water Algal sample was collected in order to identify, characterize and take them for pulp supplement. The bulk sample of algae was collected from water tank in the area of Bhavanisagar near Sathyamangalam. The sample was collected in a time interval of 15 days. The collected sample was dried (shallow drying) and stored in room temperature for further analysis.

Plate 1: Algae grown in water tanks

4.1.2. Cotton

The three different categories of waste cotton were collected from the cotton mill. The categories were based on their fiber length.

4.1.3. Humus

The waste leaves were collected from PSG College of Arts and Science, Coimbatore ant it is soaked in water and stored for pulp preparation.

4.2. Morphological characterization

4.2.1. Wet mount techniques

The algal samples were thinly sliced using a dissection needle into individual thread like substance and then placed onto Petri dish cover containing distilled water. Then these individual threads were picked up and placed onto a clean glass slide and cover slip was placed over it. Then the slides were viewed under microscope. The algae were seen as unbranched filamentous thread like structure. It is identified as *Microspora sp.,* based on the standard.

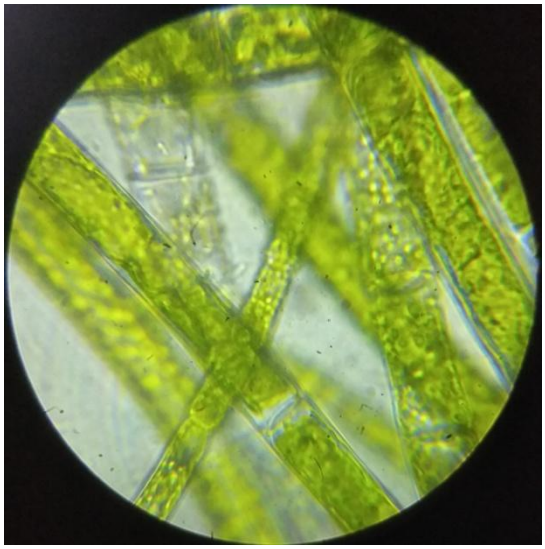


Plate 2: Microscopic observation of algae

4.2.2. Cultural characteristics

The algae were cultured into the BG 11 medium. 100ml of the medium in 250ml flask were prepared. Then 2g of samples were inoculated into the medium. It was incubated at 28ºC in algal chambers where the light and dark reactions were maintained using artificial light. After 7 days of incubation, the algal biomass was observed as bright green color.

4.3. Biochemical analysis for algae and cotton

The reducing sugar, glucose, cellulose, hemicelluloses and lignin content for algae and cotton were determined.

4.3.1. Estimation of Reducing Sugar by Dinitrosalicylic Acid Method

The amount of reducing sugars present in the algal sample is estimated to be 65% by dinitrosalicylic method. The amount of reducing sugars present in the waste cotton sample is estimated to be 80% by dinitrosalicylic method on average.

Rodrigues and Silva Bon (2011) also reported that when the amount of glucose relative to total reducing sugar was calculated it was found that 60.4% of all reducing sugar was glucose for most of the algae.

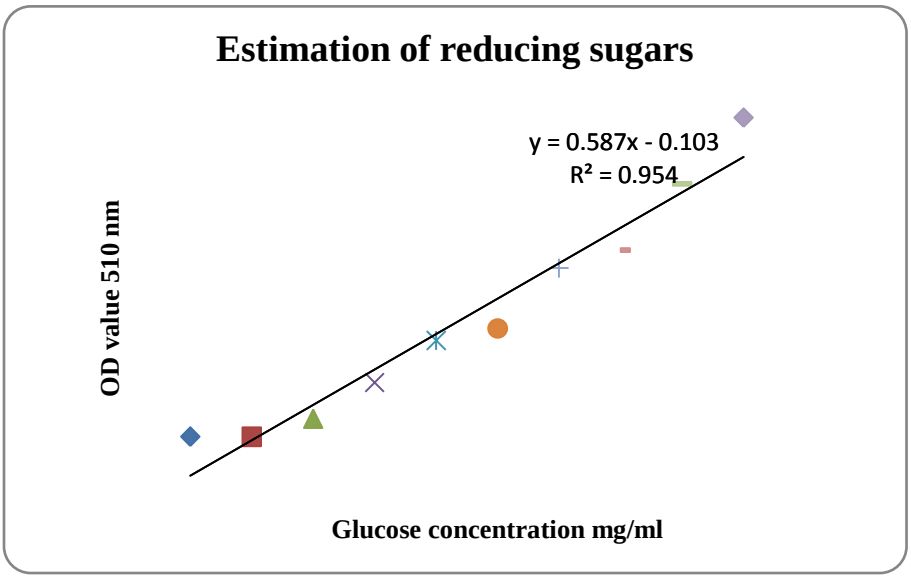


Figure 8: Standard graph of Glucose for estimation of reducing sugar

4.3.2. Phenol Sulphuric Acid Method for Total Carbohydrate

The total carbohydrate present in algae and three different varities of cotton was estimated by using phenol sulphuric acid method. In hot acidic medium glucose is dehydrated to hydroxyl methyl furfural. This forms a green colored product with phenol and has absorption maximum at 490 nm.

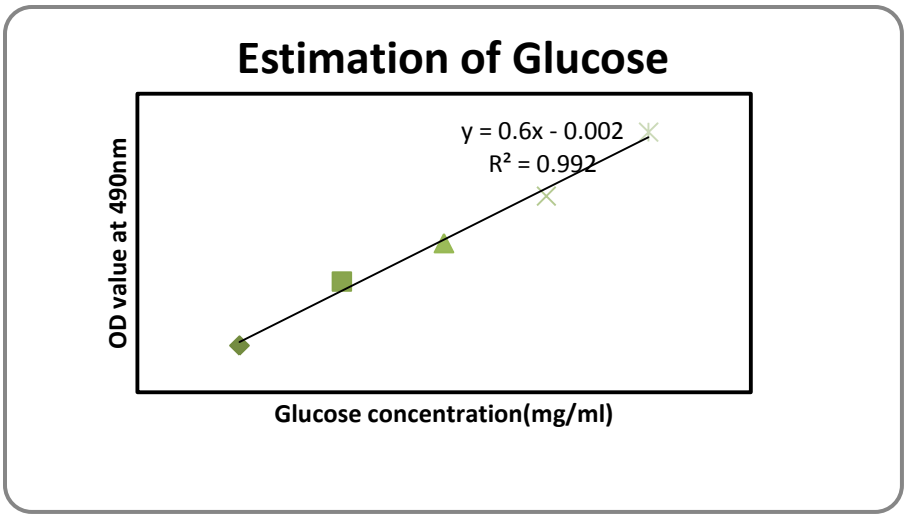


Figure 9: Standard value of glucose for estimation of total carbohydrate

Calculation

Absorbance corresponds to 0.1ml of the test = x mg of glucose

100ml of the sample solution contains = (x/0.1) × 100 mg of glucose

= % of total carbohydrate present

The total carbohydrate in algae was estimated to be 0.49mg/ml. The total carbohydrate present in waste cotton sample was estimated to be 0.7% on average.

4.3.3. Cellulose estimation

The glucose concentration obtained was used to determine the cellulose concentration.

%w/w cellulose content = 0.9/0.96x C 1 x (V/M) x α x 100

The cellulose content in algal sample was estimated to be 4.6g/litre. The cellulose content present in waste cotton samples is estimated to be 7% on average.

Ververis *et al.,* (2006) reported that *cellulose* content of algal biomass was found to be 7.1%.

4.3.4. Hemi cellulose estimation

The glucose concentration and the reducing sugar concentration were used to estimate the hemicelluloses content.

% w/w hemi cellulose content = 0.88/0.93 x C 2- C 1 x (V/M) x α x 100

The hemicellulose content in algal sample was estimated to be 15.13g/litre. Ververis *et al.,* (2006) reported that the hemicelluloses content of algal biomass was 16.3%.

4.3.5. Lignin and Ash content

The solid residue obtained after treatment of sample was dried at 105°C for 24 hours and weight (W1). The residue was then transferred to a pre-weighed dry porcelain crucible and heated at 600°C for 5 hours. After cooling down, it was weighed (W2) and ash content (%) was determined. Acid insoluble lignin was then calculated by the difference (W1-W2).

Ash content = 1.34 (W2)

Acid insoluble lignin = (W1 – W2)

= (2.5 – 1.34)

= 1.16

Ververis *et al., (*2006) reported that the ash content and acid insoluble lignin of algal biomass were 1.34% and 1.16%, respectively. The ash content of cotton was found to be 7.6%.

4.4. Optimization of algae

The effect of different parameters on the growth rate of algae was assessed. The culture conditions which controlled for the algal growth were nutrients, light and pH.

4.4.1. Culture medium/Nutrients

The algal growth was monitored by culturing it in Blue Green 11 and Bold Basal medium. The cell mass was separated through filtration and then weighted after blotting the excess water. The best algal growth rate was observed in BG11 medium.

Plate 3: Optimization of Algae on different medium

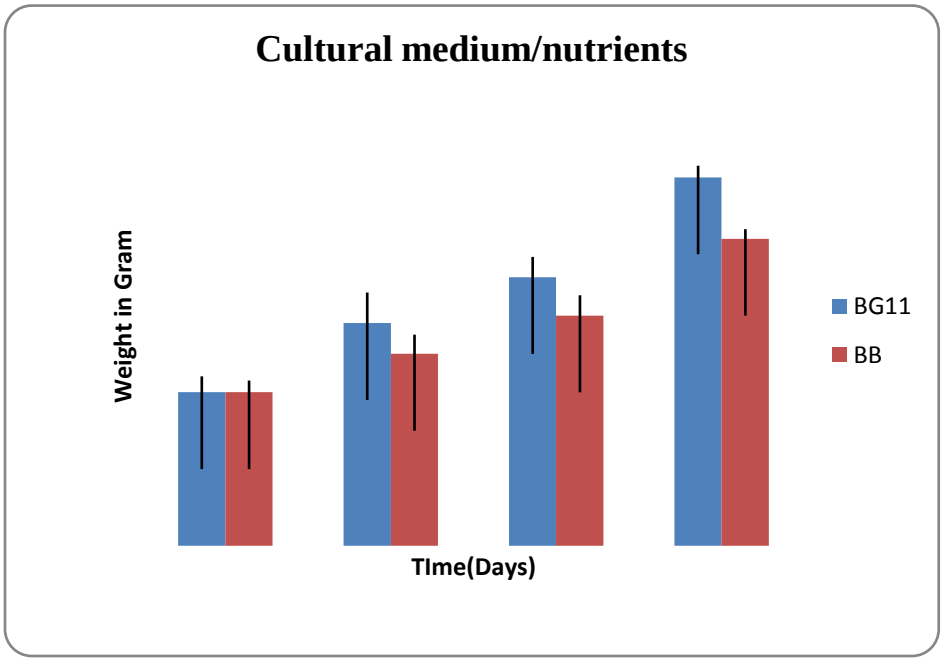


Figure 10: Effect of different media on Algae

The similar work was done by Munir *et al.*, 2015. From which he concluded that the algal samples grows better in BG11 medium as compared to BB medium.

4.4.2. pH

The algal growth on various pH ranges (4, 5, 6, 7, 8) in both culture media. The cell mass was separated through filtration and then weighted after blotting the excess water. The best algal growth was observed on pH 6-7 in BG11 medium.

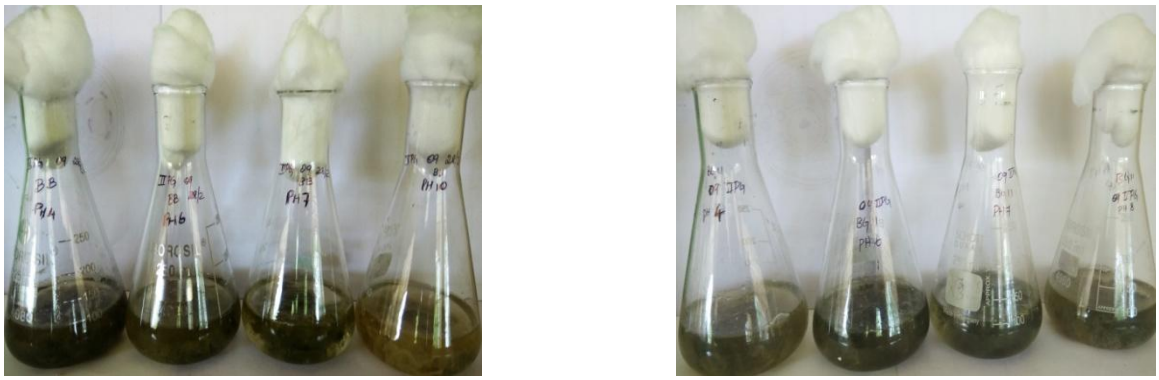


Plate 4: Optimization of Algae on different pH in different medium

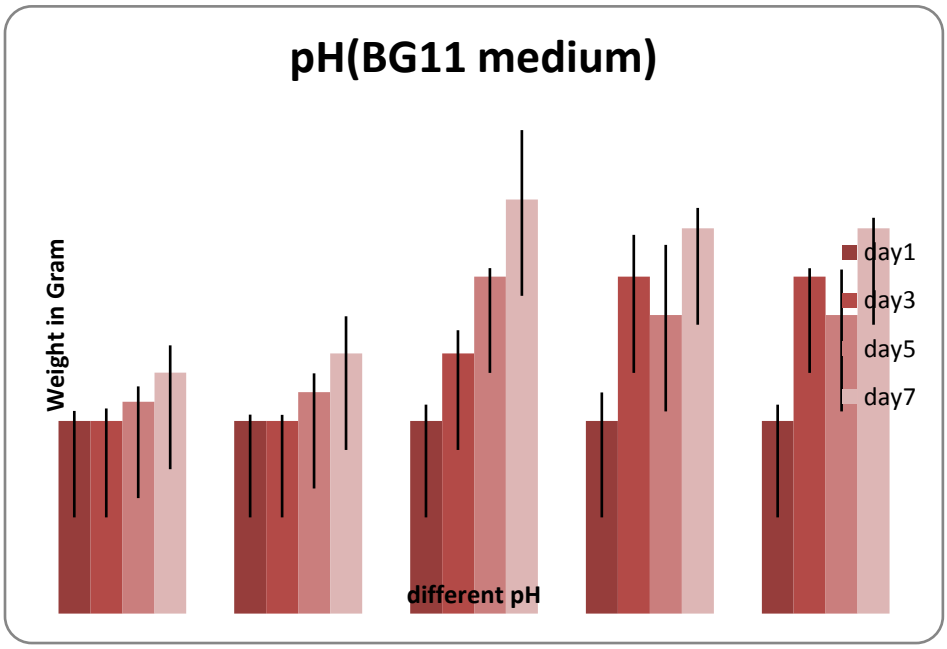


Figure 11: Effect of pH on Algae on BG11 medium

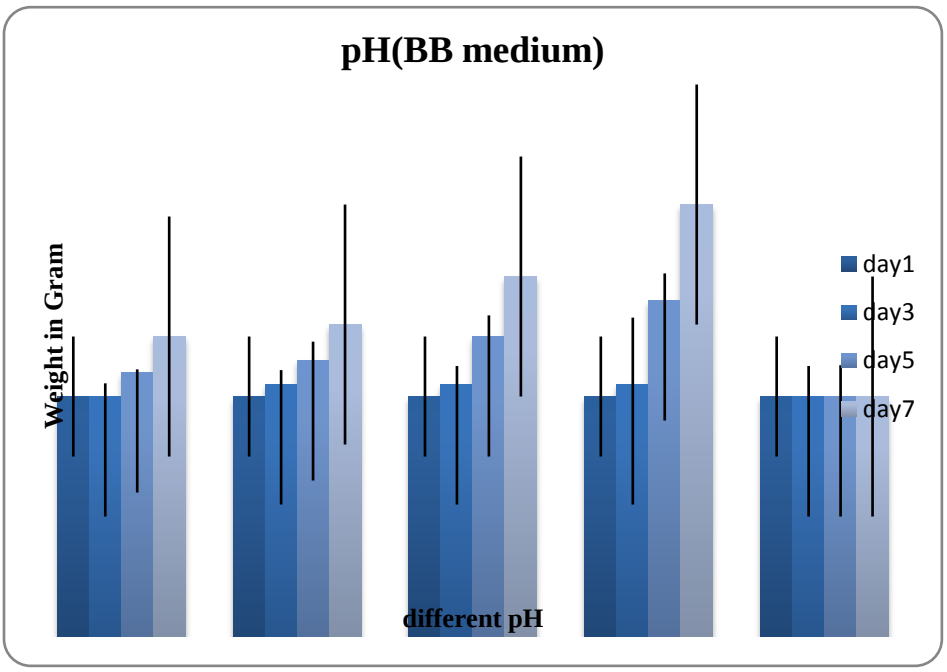


Figure 12: Effect of pH on Algae on BB medium

The findings of our work are in agreement with those publicized by Zhu (2010) whose results depicted that algae grew best in media adjusted with an initial pH of 6 and thus algae have shown fastest growth when the pH was near 7. He also concluded that in growth medium with a pH of 5.0 and 9.0 algae did not show apparent growth, thus the desired pH for growing algae is 6-7.

4.4.3. Light

Algal cultures were placed out door in open sunlight, inside the lab near window and under artificial light provided by fluorescent tubes. The effect of light on the growth of algae was calculated by measuring the algal biomass. The BG11 medium which incubated in artificial light showed the best algal growth.

The similar work was done by Newsted, 2004. From which he concluded that under artificial light, the algal biomass exhibits better growth.

Plate 5: Optimization of Algae on different light source in different medium

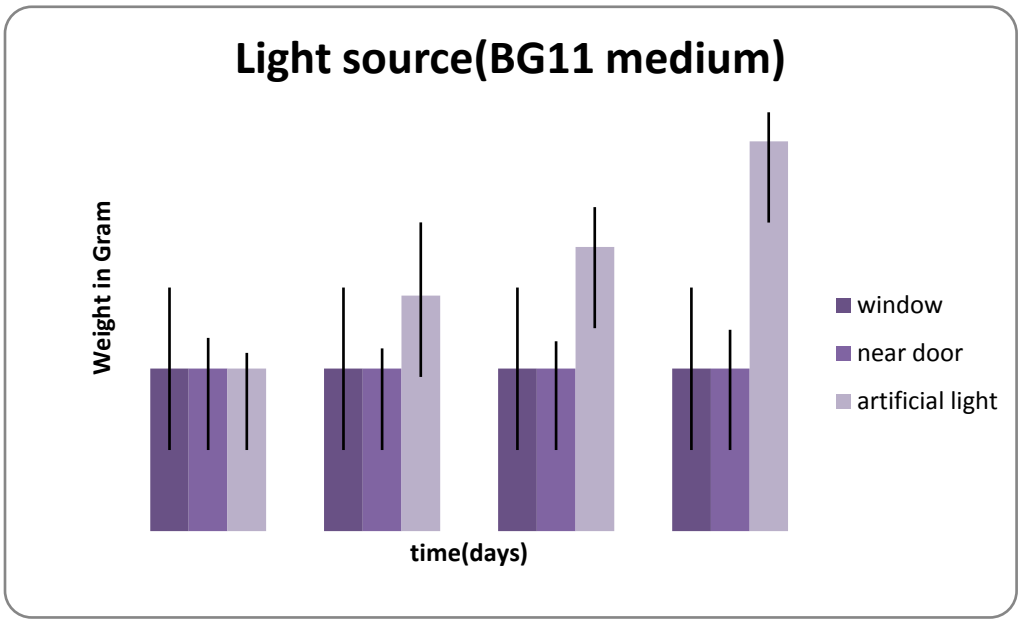


Figure 13: Effect of light source on Algae on different medium

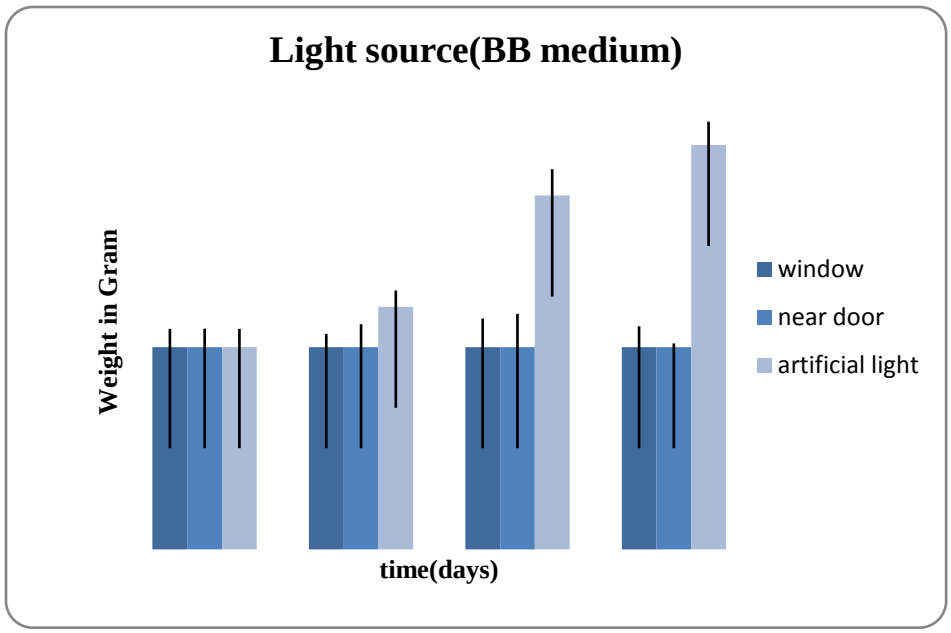


Figure 14: Effect of light source on Algae on different medium

4.5. Algal cultivation and optimization in tanks

10g of Algae were inoculated in 500 liter water tank along with 250g of glucose. The glucose was provided in the time interval of 5 days. After 10 days the algal biomass was weighed. When compared with normal algal growth (4 kg) in 500L of water without the presence of glucose, the algae grown with glucose (6 kg) showed 2 kg increases in the biomass production. This shows that when glucose was given as a substrate for the growth of algae it showed a better yield of biomass.

Plate 6: Algae grown in water tank along with glucose

Hong-Yu Ren *et al* (2013) also reported that when glucose was added as a substrate there was increase in algal biomass yield. They said that when the glucose concentration increased from 5 to 30 g L-1, the biomass sharply increased from 1.58 to 4.12 g L-1, while further increase of glucose to 100 g L-1 led to the decrease of cell concentration. This indicated that excessively high or low glucose in the medium had inhibitory effect on the cell growth.

When compared with Hong-Yu Ren *et al* (2013) where they used 30g of glucose/l but in the above research 0.5 g of glucose / litre showed greater yield.

4.6. Paper making

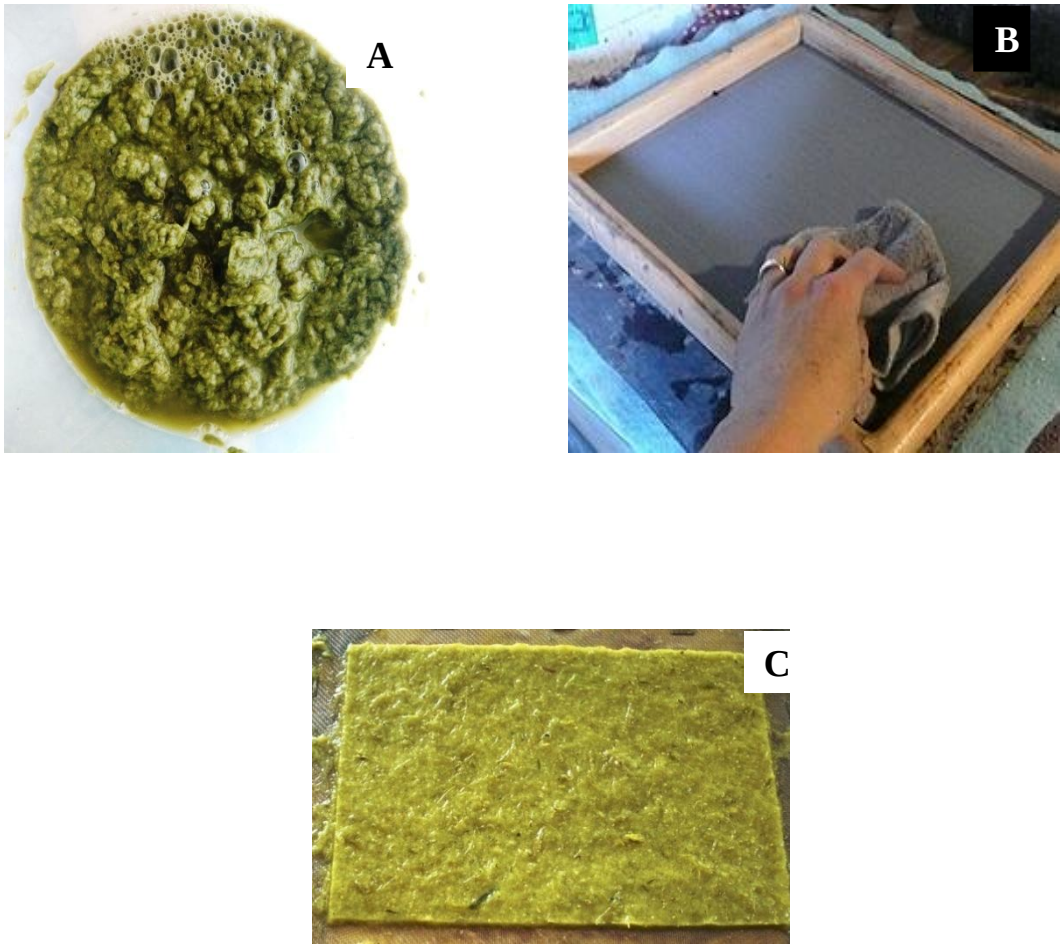
4.6.1. Control

The paper pulp was obtained from paper mill- Gajendra paper and boards limited, kethanur. The obtained pulp was added with 1000ml distilled water and poured in a tub for making paper. The consistency of the pulp in the tank was maintained constant all the time. Once the sheet is formed, the frame was separated, pressed and dried.

4.6.2. Algal paper

The collected algal sample was dried and blended along with humus, waste cotton and waste pulp in different ratios - 1:1:1:2, 1.5:1.5:1:1, 2:1:1:1, 3:2:1:1, 4:2:1:1 and 5:2:1:1. 150ml of the blended pulp was added with 1000ml of distilled water. Then the pulp was poured into the tub and the mixture is mixed thoroughly. The pulp was made into paper by the same procedure as of control.

Among all the different ratios, 5:2:1:1 was equally good as control paper. In this ratio 50% of algal sample was used which shows that algal biomass can be used as an alternative for paper making.



A- Blended algal pulp B- Making of pulp into sheets C- drying of sheets

Plate 7: Algal pulp preparation and papermaking

4.7. Quality analysis of the produced paper

Testing the quality of the paper by physical and optical properties: Basis weight, bursting strength, cobb factor, and ply bond factor were checked. The paper quality was checked in paper mill-Gajendra paper and boards limited, Kethanur.

4.7.1. Gram per square meter (GSM)

The term GSM refers to the **substance weight** of paper, relating to an area of paper that remains constant, irrespective of sheet size, expressed as grams per square meter. The higher the GSM number, the heavier the paper.

35gsm to 55gsm: This is very thin paper indeed. Most newspapers will commonly be printed on this paper thickness.

90gsm to 100gsm: This is the weight of most household printer paper. The stuff you might pick up in packs of 500 sheets at the office depot.

120gsm to 140gsm: This GSM range covers the paper thickness of most posters you're likely to find on pub walls etc. Paper with this GSM is sturdy enough to withstand a bit of wear and tear. It's also the thickness of low-cost flyers you might have had posted through your front door by minicab companies and the like.

210gsm to 300gsm: Moving onto premium flyers now. This GSM range will cover most of the sturdy printed flyers you get given in the high street. This paper stock range is approaching card but will still have a bit of a bend when held with two fingers. Think of the magazine covers you see on the racks at newsagents.

350gsm to 400gsm: This GSM is essentially cards. It will stand up under its own weight and is most commonly associated with premium flyers and business cards. As well, it is likely to be the stock that high-quality wedding invitations are printed on.

The produced algal paper had the GSM value of 215gsm which is more or less similar to control paper (220). This shows that the paper produced is suitable for premium flyers.

4.7.2. Bursting strength

Bursting strength tells how much pressure paper can tolerate before rupture. Bursting strength is measured as the maximum hydrostatic pressure required to rupture the

sample by constantly increasing the pressure applied through a rubber diaphragm on 1.20 - inch diameter (30.5 mm) sample.

The bursting strength of the produced algal paper was found to be 1.65 KPa m^2/g. Mukherjee and Keshri (2018) also reported that the bursting strength of the algal biomass was 1.7 KPa m^2/g. The alternative use of algal pulp has increased the tensile strength due to the acquired burst index.

4.7.3. Cobb factor

The Cobb test determines the amount of water absorbed into the surface by a sized (non-bibulous) paper, paperboard, and corrugated fibre board paper or paperboard sample in a set period of time, usually 60 or 180 seconds (Cobb60 or Cobb180). Water absorbency is quoted in g/m2. The water absorbency of a material can have strong influence on printability and the setting rate of water based adhesives.

The absorption rate of the algal paper was 0s. The paper absorbed water soon as it was a handmade paper and there was no use of any chemical for making of paper and no waxing was done.

4.7.4. Ply bond factor

The Internal Bond Strength of paper or paperboard (also known as Ply Bond Strength or Z Directional Strength) is the ability of the product to resist splitting when a tensile load is applied through the paper's thickness i.e. in the Z direction of the sheet. The interlayer strength of the paperboard, measured on Scott Bond Tester, expressed in J/m^2.

The obtained results were 212 J/m^2. This indicates that the produced paper is at the range of coated cover paper. The standard procedures are explained in TAPPI T 403 & T569 & SCAN P80.

Table 2: Standard Scott Bond Values (TAPPI T 403 & T567 & SCAN P80)

Typical Scott Bond Values	
Grade	J/M
Cover paper	125-230

Offset paper	240-290
Xerographic paper	220-400
Coated cover paper	200-315
Coated text	240-365

4.8. Application

The algal pulp was made into paper and they were made used for different applications.

- Algal Paper was made egg tray

Plate 8: Egg tray

- It was made into paper thread cone.

Plate 9: Paper cone

- It was made into gift boxes

Plate 10: Gift box

DISCUSSION

The present research work is on searching for paper pulp supplement from fresh water algae. The samples were collected from different locations and the algae were identified based on the Morphological characteristics given in the book entitled “Structure and reproduction of algae” by F.E. Fritsch (1935).

The identified algae were analyzed by using biochemical techniques like DNS and Anthrone method for Cellulose and Hemicellulose, Ververis *et al* for Lignin, Lipid and Ash content. The Cellulose and hemicellulose contents of algal biomass were 7.1% and 16.3%, respectively, For all materials, lignin and ash content was 2% or lower, rendering them suitable for use as paperpulp supplements. The addition of algal biomass to paper pulp increased its mechanical strength significantly. However, brightness was adversely affected by chlorophyll. The addition of citrus peels in paper pulp had no effect on breaking length, increased bursting strengthand decreased tearing resistance. Brightness was negatively affected at proportions of 10%, because citrus peel particles behave as colouredpigments. The cost of both materials is lower than that of conventional pulp, resulting in a 0.9–4.5% reduction in finalpaper price upon their addition to the pulp (Ververis et al., 2004)

The biochemical parameters of the identified algal biomass for the present study were estimated as, cellulose (14.05%-0.101%), hemicelluloses (9.803%-2.5%), lignin (1.346%-0.13%), Ash content (0.965%-1.21%), lipid (1.9%-0.2) etc are done to check for their suitability in paper production.

The pigments of algal samples were extracted using three different solvents.The extraction was complete because when compared to other compound chlorophylland carotene pigments are non-polar organic substances. Chlorophyll and carotenesdon’t

dissolve in water, but they dissolve crazy in organic solvents. Chlorophyll a was higher in *Caulerpascalpeliformis*. Chlorophyll a was lower in *Kappaphycus alverazzi*, red algae an exotic speciesbrought for its commercial importance. *Caulerpa scalpeliformis* has a large number of chlorophyll a when compared to *Ulva lacuta* and *Ulva reticulate*. Secondly chlorophyll b content has slight variation and significant difference within each species. The maximum number of chlorophyll b was recorded in *Ulva reticulate* and in *Kappaphycus alverazzi*, the amount of chlorophyll b is nil and this may due to cell wall rigidity. Total carotene was determined the highest in *Ulva reticulate* and there was less number of total carotene pigments in the algae (P. Kumar*et al.,)*

Most importantly a solvent plays a major role in the extraction process ofchlorophylls and carotenes. The pigments were calculated and formulated based onthe formulas of Lichtentaler&Wellburn (1983). It was determined that the solvent used were important in the determination of pigments. Ethyl acetate was considered as the best solvent for the extraction of pigments because ethyl acetate is easily miscible and the extraction was complete when compared to ethanol and acetone. In many classes of algae cellulose is the main and foremost structural element of the cell wall, though there may be remarkable variations of the fibrillary structure that exist. Most of the Chlorophyta members have layers of parallel microfibrils and species of Rhodophyta such as *Porphyra*, *Kappaphycus* constitute mannoses as the main structural elements. These solvents are large enough to break up these structure and helpful in extraction of pigments.

In the present study chlorophyll and carotenes does not dissolve in water, but they dissolve in organic solvents. The extraction of pigments from *Osillatoria brevis* using acetone showed high amount of chlorophyll a (6.24-1.02µg/ml) and chlorophyll b (5.82-1.82µg/ml). The extraction of carotene using diethyl ether ranges from 1.89-0.02µg/ml.

The addition of algal biomass to paper pulp increased its mechanical strength significantly. However, brightness was adversely affected by chlorophyll. The addition of citrus peels in paper pulp had no effect on breaking length, increased bursting strength and decreased tearing resistance (C. Ververis, 2007)

In the present study the paper produced from *Oscillatoria brevis* has less brightness and decreased tearing resistance but they have a high mechanical strength

Summary and Conclusion

Wood is a complex material consisting of flexible cellulose fibers bonded together and made rigid by a complex organic “g l u e” called lignin. Slightly less than half of the wood in the tree is actually made up of the cellulose fibers that are desired for making paper. The remainder of the tree is lignin, wood sugars and other compounds. Separating the wood fibers from the lignin is the task of chemical pulping processes. Algae have got vast application potential in the industrial, pharmaceutical and medical areas. The use of algae in paper industry could lead to a revolution as currently large number of trees is cut down in order to meet the day to day needs of paper. This characteristic of algae may be attributed to its similarity with that of the plant. They are not only similar in their mode of nutrition but also in various other properties. This could attribute for their use in paper production as a supplement for the paper pulp substituting the wood pulp.

The present study deals with the potential of microalgae as supplement for the paper pulp in handmade paper making. Thus the estimations of biochemical parameters such as cellulose (14.05%-0.101%), hemicelluloses (9.803%-2.5%), lignin (1.346%-0.13%), Ash content (0.965%-1.21%), lipid (1.9%-0.2) are done to check for their suitability in paper production. The biochemical estimations are very well suggestive of the use of algae in paper production. Thus theoretical confirmation could be well asserted based on the facts of presence of cellulose and hemicelluloses. The absence of lignin also plays a wide role in their feasibility as they make the paper less suitable by early deterioration and self-adherence. To make more suitable pulp from algae it is important to extract these substances through simple treatment techniques and use. This again leads to the rise in production cost but which could be dealt with the unwanted residues after treatment to be used as food and feed additives. This could make the residues go to another economic purpose thus reducing the waste as well the cost of the pre-treatment.

The capability of microalgae to produce pigments such as chlorophyll a, chlorophyll b and carotenoid. The microalgae pigments were extracted using three different solvents. The extraction was complete because when compared to other compound chlorophyll and carotene pigments are non-polar organic substances. Chlorophyll and carotenes don’t

dissolve in water, but they dissolve crazy in organic solvents. The solvent extraction using acetone showed amount of chlorophyll a ranges from (6.24-1.02µg/ml) in Osillatoria brevis and chlorophyll b ranges from (5.82-1.82µg/ml). The solvent extraction using diethyl ether showed amount of total carotene ranges from (1.89-0.02µg/ml). The application part of the study deals with use of the algae in handmade paper making by supplementing different concentrations.

- The Algal sample was collected from the water tank in the area of Bhavani near Sathyamangalam. The waste cotton sample was collected from the cotton mill.
- The collected algal samples were identified and characterized based on their morphological/microscopic features. The algal sample was identified as *Microspora sp.*
- The reducing sugar, carbohydrate, cellulose, hemicellulose, lignin and ash content were estimated for algal sample and cotton sample for making paper. The amount of reducing sugar, total carbohydrate, cellulose, hemicelluloses, ash content and lignin content present in algae were estimated to be 65%, 0.49%, 0.46%, 1.51%, 1.34% and 1.16%, respectively. The amount of reducing sugar, total carbohydrate, cellulose and ash content present in cotton were estimated to be 80%, 0.7%, 7% and 7.6% respectively.
- The algal sample obtained was optimized under laboratory conditions. The effect of different parameters on the growth rate of algae was assessed. The different parameters used for assessing were nutrient conditions, light and pH. The algae showed better growth in BG11 medium incubated with artificial light in the pH ranges from 6-7.
- The algae were cultivated in 500L water tank with 250g of glucose in a time interval of 5 days. After 10 days of incubation, the increase in algal growth was observed. There was increase of 2 kg of algae when compared to algae grown without glucose.
- The paper pulp was obtained from paper mill - Gajendra Paper and Boards limited, Palani. This pulp was made into paper sheets which are considered as control paper.
- The algal pulp was prepared with algae, cotton, humus and waste paper in different ratio. The algal paper sheets were made and it was dried. The algal paper with 50%

of algae is equally good as control paper which shows that algal biomass can be used as an alternative for paper making.

- The quality of the produced paper was analyzed in paper mill - Gajendra paper and boards limited, Palani. The Algal paper showed GSM value of about 215gsm, the bursting strength was about 1.65 KPa m^2/g, the cobb was nil and the ply bond factor obtained was 212J/m^2. This shows that the paper obtained is of good quality and can be used for various purposes.
- The produced paper was used for different applications like egg tray making, thread cone making and gift box making. They showed effective results over the applications in laboratory scale. In future they will be taken into large scale and various other applications will also be met.

Papers Presented/Published and sent for Publication:

1. Algae in Production of Paper Pulp and formulation of simple media for Microalgae cultivation using waste water. (Seminar Proceedings. National Seminar on Prospecting for Today's World. 8th January 2014. Department of Microbial Technology. Bharathiar University. Coimbatore).

2. Achuth Jayakrishnan, Subhashree RCM2, Krishnaveni N3. Nov. 2016. Algae As Promising Supplement for Paper Pulp – An Ecofiendly Approach to Save Trees. Fifth International Conference on Sustainable Utilization of Tropical Plant Biomass : Bioproducts, Biocatalysts and Biorefinery (SutB4) organized by Department of Microbiology, TNAU,C Coimbatore (Poster Presentation. Nov 2016).

3. Achuth Jayakrishnan, Subhashree RCM, Krishnaveni N. Exposition and Categorization of Cellulose, Hemicellulose content existing in Microalgae, Macroalgae isolated from ETP and Marine Water sources and their Pigment Extraction. (Communicated and sent for publication to International Research Journal of Biological Sciences)

4. Krishnaveni. N, S. Hemamala, N. Kannan. Detection of Algae from Sewage Treatment Plant. (Communicated to PSGCAS SEARCH Journal).

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