Course on Industrial Biotechnology Professor Debabrata Das Department of Biotechnology Indian Institute of Technology Kharagpur Lecture 46 Module 10 Spirulina Production

Welcome back to my course the Industrial Biotechnology this lecture we will cover something new product that is the another type of single cell protein that is the spirulina because algae nowadays lot of use for different purposes because we have algae can be used as a food as a pigment as a for medicinal purpose it has tremendous potential that we have in the market.

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So I want I have chosen that one product is largely market and the research work has been carried out for quite some time that is the spirulina, spirulina production that we know and the spirulina represents a group of blue-green algae what we call is cyanobacteria refers to dry biomass obtained from the species of Arthrospira Platensis and Arthrospira maxima this is the name of the organism that is used.

And it can be consumed by human as dietary supplement and can be used animal feed because it has the high nutritional value. The use of spirulina dates back 16 century and used the Aztecs and other other Mesoamerican as food source. Apart from being used as a food source this primitive life form is responsible for producing oxygen in the atmosphere, allowing the life form to develop.

Now here I want to point out one very interesting thing that we have one project on this for algae aglal production that is food, feed and and fuel production and this is kind of window Denmark project we had and we work in details on this project and we have we have we have collaborator in India we have collaborator from Denmark. In India we have this Indian Agricultural Research Institute they have national centre for xyno bacteria they they they walk with us and also in the Mudurai Mudurai we have the antenna there is a company they produce sub spirulina in the large scale.

So we have a very good experience and walking on this particular area and when I we visited the Mudurai we can remember that we visited that my collaborator took us in some village and we and the villagers, they they have they presented the use of for spirulina in the form of some drama and they shows that how it is it it has the give the benefit to the day to day life not not only to has have the nutritional supplements but also some kind of health problem like gouts and other things they recovered by using this spirulina. So this is very so it is a very useful material that is that can be used for human consumption.

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And benefits is that it is it has the 50 to 70 percent of protein more than the beef and chicken and soybean, 8 essential and 10 nonessential ammonia acids are present there. Gamma linoleic acid and beta carotene linoleic then arachidonic acid then vitamin B12, iron, calcium, phosphorous and phycocyanin a pigment protein complex that is found only in the blue-green algae. So this this is not only used as a food supplement but used as a natural colour as a source of natural colour there is the major advantage of this it it also give the help to produce some kind of natural colour which is used for different purpose it does not have much of this harmful effect to the human beings.

And it is used as a food supplement for the aquaculture, aquarium and poultry industry that it finds very useful material for this purpose. Spirulina is a used as food dietary supplement and nutritional supplement and natural health product because I told you it has some that the medicinal value so it can be used as a health product. Some of its components are also used as food colouring agent in Japan where this is the natural colour this is usually permitted we know most of the inorganic colour that is not good for our human health.

So the certain cosmetic also used the spirulina as a active ingredient because because some some cosmetics particularly I can tell you lipsticks that is the woman that mostly use, if it is made of synthetic material sometimes it has some effect on the skin and not only skin that helps the health to a great extent, so natural colour gives better because it does not have much of harmful effect to the human beings. So this has lot of applications as per human beings are concerned.

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Now one important aspect I can tell you that if it looks very nice when you see it under microscope it looks very nice you can easily visualize from here the arthrospira the species is a free floating filamentous cyanobacteria characterized by cylindrical multicellular trichomes

in an open left hand helix, can you see that that how it how it that helical formation is there it is it is like this.

So you can you can see, if you if you see under microscope it looks very nice and this occurs naturally in tropical and subtropical lakes with a high pH and high concentration of carbonate and bicarbonate because you know that this, this is a mostly the auto tropic organism and they fix the carbon-di-oxide in the form of some organic molecules. So the availability the largest commercial producer of spirulina are located in United States, Thailand, India, Taiwan, China, Bangladesh, Pakistan, Burma what you call Myanmar and Greece and Chile.

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The cost of production of spiruluna dietary product in India ranges from 600 to 100 rupees per kg of the product and you can see how this is this is grow in the open pond. This is a open pond it can grow like this.

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Now mode of growth this tiny green spiral coils harvest the energy of the sun and growing as a growing a treasure of bioavailable nutrient, so this is like this autotrophic growth I told you this use the carbon-di-oxide we have we have three type of growth, we have autotrophic growth, we have mixotrophic growth, we have heterotrophic growth.

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Now if you look at the autotrophic growth an organism that is able to form the nutritional or nutritional organic substances from the simple inorganic substance like carbon-di-oxide then we call it autotrophic, autotrophic means they use the carbon-di-oxide as a body building materials. The heterotrophic means an organism derived from the nutritional requirements from the complex organic substances, they use the complex organic substances as the for the nutritional requirement, so this is called heterotrophic.

And mixotrophic, an organism that can be used used a mix of different sources of energy and carbon instead of having the single trophic mode of the of the continuum from complete autotrophic at at one end and to heterotrophy in the other end. So they can use both carbondi-oxide and the organic organic compounds for their growth and metabolism that is we consider as a mixotrophic.

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Now now here you see that this is the autotrophic this is carbon-di-oxide is mixing here and producing micro algae the mixotrophic we use the different nutrients that inorganic and carbon-di-oxide also we use all this that is why I am saying in mixotrophic and here we use the soluble organics that is why we call it heterotrophic organism so this is how it can be explained that in a systematic manner.

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Metho	ods of cultivation -	photobior	eactors
			ل دری درما
a plate	b tubular	c annular	d plate airlift
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So so methods of cultivation is required the different type of photobioreactors, photobioreactors means they utilize the sunlight for their growth metabolism we have we have different types of photobioreactors, we have plate type of plate, tubular, annular and the plate airlift reactor that we have so plate airlift reactor that we have. Now your plate type of heat exchanger you can see we can spurge carbon-di-oxide at the bottom and you can purge light to both the side and we that algae can grow here the tubular we can pass the liquid like this it comes out like this and we put the light source in the may be in the one way or two ways whatever you have.

In the annular reactor is very we find very efficient but very difficult to handle because we put the light source at the middle and liquid it goes to the annular space when it goes the light will be equally distributed in the surrounding areas and this is this is plate airlift that is how it is it is what can so that it kind of recycle things that you can see that take place inside the system.

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Now this closed bioreactors we have we have two types of photobiorecators that is largely used for the algal biomass production one is open pond another is closed bioreactor, open when we use open there is every possibility of getting contamination from the because because other other algae we present in the atmosphere, they may grow in a in a as a contaminants other not only other bacteria also can grow that affects this particular fermentation process and that is that is undesirable but again that depends on the quality of the media that we use.

Now if we use the media which will not allow other much much of the contaminant to grow then you can use the open pound otherwise it is very difficult to grow difficult to use this open pond for the growth of a specific type of algae on the in the open (())(12:26). Closed close bioreactor we have close environment and we have their we can allow one particular type of algae and grow in the close environment so that the rate of growth of the organism will be very high and if we if we look into the cost aspect obviously the in case of open pond the cost will be much less operational cost also and installation cost both will be less whatever close reactor your both the cost will be little bit higher.

Now this is like this the closed photobioreactors looks like the plate type and I I this is this can be problem in a manner so that as your this is usually located at the at the roof as your as the sun shifted its position this automatically this plate shifted in a manner so that most of the light energy can be used for the growth of the their algae. Now this is we have we have we have both airlift as well as the bubble column reactor then we can use this column

for the growth of the algal cell. This is kind of tubular reactor that also largely used for the algal biomass production.

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Now in India as as I told you that we we I have the collaboration with with antenna there is the one group that they have the collaboration with the sister concerned of one company from Switzerland and they they they they have this open raceway pond, you can see how it looks. We got different type of raceway pond is there and they they have some kind of protection at the top so there is some dirt and other thing should not fall on the on the pond and this is the this is this is located in the Madurai in the southern India of southern part of India.

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	Commercial pro	oduc	ers of Spirulina	3
Name of company	Location			
Earthrise Farms Cyanotech Corporation Myanma Microalgae	Calipatria, California (USA) Kallua Kona, Hawaii (USA) Yangon (Myanmar)	S. No	Manufacturers Name	Location
Biotechnology Project Hainan DIC Microalgae	Hainan (China)	1	Ramsun Rasayanics	Karnataka
Nao Pao Resins Chemical Solarium Biotechnology	Tainan, Taiwan (China) La Huayca (Chilo) PireTung County, Taiwan (China)	2	Om Spirulina Unit	Andhra Pradesh
DIC LIFETEC	(Japan) Bannong, Raiburi (Thailand)	3	Parry Nutraceuticals	Tamil Nadu
Siam Algae Ballarpur Industries TAAU Australia	Bangsaothong (Thailand) Nanjangud, Mysore District (India) Darwin, Northern Territory	4	Dabur group's Sannat Products	Tamil Nadu
Sosa Texcoco	(Australia) Lake Texcoco (Mexico) Fiar Oscael)	5	NB Laboratories	Mumbai
*Adapted from Habib et al. (200 Sanchez et al. (2003)	08), Ciferri and Tiboni (1985) and		Selvendran, D. (2015),	, Tradeindia.com

Now the commercial producer of spirulina there are different companies that we have in India as well as in the foreign countries we have this is the this is the giving here, the Cynotech corporation, the Myanma microalgae, Biotech Project there is so lot of companies that have been different places in the world and India we have this company Ramsun that is this Rasayanics company that is in Karnataka, Om Spirulina Unit in Andhra Pradesh, Parry Nutraceuticals that is Tamil Nadu, Dabur Group of Sannat Sannat products that is Tamil Nadu, NB Laboratories Mumbai. So different companies in India they produce this spirulina in large scale.

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And the spirulina is produced by industry in a large scale in the raceway ponds adopted by the mechanised agitated, filtering and drying system with relatively higher capital investments. In order to cultivate spirulina one one must produce all necessary elements which will be will permit the culture to absorb and utilize this element. This involves the range of suitable temperature, illumination to provide the energy needed for photosynthesis, water and nutrients nutrient. Usually usually grown in autotrophic condition in the open environment, so this is this is the feature that we have for the industrial spirulina growth.

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	Industrial production of Spirulina
•	Spiruling producing farms choose tropical region so that the required temperature range o $30 - 37^{\circ}$ C in day time and 25- 30° C in night time obtained.
Re	quirements
	Rich source of water
	10 h of illumination (minimum)
	Low humidity
	Low wind
	Regarding nutrient source requirement, one must supply organic / inorganic sources of carbon, nitrogen and minerals.

And this spirulina produce producing farm choose the tropical region so that the requirement of temperature range 30 to 37 degree centigrade in the day time and 25 to 30 degree centigrade at the night time. The requirement rich source of water that is water is main source for this cultivation, water is required, 10 hours illumination because again if you look at some western country that there their illumination is drastically reduced in some particular season.

So in in the tropical country is suitable the reason is that that illumination is mode as compared to some of the western country. So here that is why we find that India is a very good place for the cultivation of the algae and 10 hours illumination minimum required for the for the growth of the algae, now low humidity, low wind, regarding the nutrient source one must supply the organic and inorganic sources of carbon, nitrogen and minerals because I told you that whenever we grow any kind of microorganisms we require proper carbon source nitrogen source minerals and vitamin.

So whatever things is required that we might have to (())(17:46) the use the pure material but we can use some cheaper source so that the food supplement can be given in a way so that your your organism growth can grow in a proper way.

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	Tank construction for spirulina cultivation
•	Building Spirulina cultivation tank is very important since many parameters are to be considered in tar construction to ensure the efficient Spirulina culturing.
•	Any water-tight, open container can be used to grow Spirulina, provided it will resist corrosion and be non-toxic.
•	Its shape is immaterial, although sharp angles should be avoided to facilitate agitation and cleaning.
•	Spiruling can be grown in tarpaulin (transferable) or permanent cement tanks.
	Most current commercial farms over the past 30 years have been designed with shallow raceway pon circulated by paddlewheels.
•	Ponds vary in size up to 5000 m ² (about 1.25 acres) or larger, and water depth is typically 15 to 25 cm
•	They require more capital investment than lake farms, and operate at higher efficiency and quality control.
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And tank construction for spirulina building spirulina cultivation tank is very important since many parameters have to be consider in tank construction to ensure the efficient spirulina culturing. Any water tight open containers can be used to grow spirulina provided it will resist the corrosion and be non-toxic, its shape is immaterial, although the sharp angle should be avoided to facilitate the agitation or cleaning.

The spirulina can be grown in tarpaulin the transparent or a permanent cement tank that then most current commercial farms over last 30 years have been designed with shallow raceway pond circulated with a paddlewheels because I shall I shall show you the raceway pond raceway pond is very easily we can explain that raceway pond is very nice.

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It is like this we have so we can so we can we can have a paddle here we can we can put a paddle here with this help of this paddle we can we can rotate this so that with the help of hand or with the help of pump we can rotate it so as as you rotate the liquid will flow this way and it will go like this it will keep on rotating like this.

So the pond pond vary in size 5000 square meter that is 1.2 acres or larger, water depth typically is 15 to 25 centimetre here I want to point out that we did some experiment in our lab we find out that if the because I was talking about the flat panel reactors like this you have seen the flat panels and question comes if you have light source here, question comes this is light source and what should be the thickness up to which thickness your light can penetrate and help for the growth of the microorganism and it has been observed that we know research work we observed that that depth of 3 centimetre is very good for the growth of the organism so this we shall have to keep in mind.

But here in case of this raceway pond they they found that the typical depth depth of the water that should be 15 to 25 centimetre, so and they require more capital investment than the lake farm and operate higher efficiency and quality control.



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So this is the raceway pond I told you how it looks that is something similar to you can see how it is that they are building out the raceway pond. They have a they have a partition wall here and this is one one tank 18 square meter produces approximately 150 gram of spirulina per day this is kind of raceway pond that they have been constructed.

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Now this is the integrated spirulina farming that that how it works this is outdoor pond that that is look at it like this the tube system, there are closed reactors that we have so both the things we can use and then then this which comes to the this the harvesting plant. Here I want to want to tell one is a very important thing that that in the algae it has been observed that 20 to 30 percent cost is spent for harvesting purpose, harvesting that means the the separation of the cells from the liquid and since the size of the algae is very not very big size.

So as a filtering you have to take the things through the filtrating process and and that is so and we find that if you use the natural settling process that it is the you have to use the special technique that separation technique so that your your your your algae can settle down very easily. We found the electro population technique is very effective to for the separation of the algal cells.

Now this harvesting plant we take it out then we dry this product and you know we can extract what the plants it can use the fish pond and product plant. So it can be marketed in different different way dry spirulina powder or crystals it can be used in the form of tablets or finished goods natural colour, vitamin, minerals for pharmaceuticals, decolouring coloured protein and also it can be used as fresh fish and shrimp and prawn products for the growth of the product has been used.

So it has if you look at the integrated form is very useful that we we can we use the carbondi-oxide present in the atmosphere we can fixed in the form of algal cell mass and then it can be used for different purpose. So we have resources then growth then harvest then processing and then we have the product this is the steps involved.



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Now flow chart for spirulina production we have the nutrient salt we have 600 metre depth at ground water, we have cultured medium, then we run run pond with agitated, then we have collection. This was concentrate dewatering, spray drying, sewing, quality inspection, spirulina powder, pressings, spirulina tablet, packaging and storage here also powder you can do the packaging and storage and this is algae filament spirulina that propagation first second tier we can we can do that and we can have this product this is how spirulina that production takes place in the industry.

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	Carbonate	2800 mg L-1	
	Bicarbonate	720 mg L-1	
	Nitrate	614 mg L ⁻¹	
Media composition for	Phosphate	80 mg L-1	
spirulina cultivation	Sulfate	350 mg L ⁻¹	
	Chloride	3030 mg L ⁻¹	
da.	Sodium	4380 mg L ⁻¹	
-	Potassium	642 mg L ⁻¹	
	Magnesium	10 mg L ⁻¹	
	Calcium	10 mg L ⁻¹	
	Iron	0.8 mg L ⁻¹	
	Total dissolved solids	12847 mg L ⁻¹	
	Density @ 20°C	1010 g L ⁻¹	
	Alkalinity	0.105 N (moles strong base L ⁻¹)	
	pH @ 20°C	10.4	
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Now spirulina we required very media composition you can see most of the minerals is contains that carbonate, bicarbonate, nitrate, phosphate, sulphate, chloride, sodium, potassium, magnesium, calcium, iron, total dissolved solid, density, alkalinity and that temperature 20 degree centigrade usually preferred that is required that.

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Now media media for spirulina cultivation, the spirulina usually grow in alkaline pH this is brackish water then the cultured medium provides all essential material for the growth of spirulina then it composed of sodium, carbonate this sodium carbonate also used as a carbon source, a source of nitrogen, phosphorous and iron and trace metals. Only the food grade chemicals are used that the choice of chemicals because if we do not use the food grade chemicals then this can be used cannot be used as a food product.

So choice of chemicals and concentration depends on the cost and compatibility of the chemicals with the raw materials. The cost of the nutrients accounts to 15 to 25 percent of total production cost.

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Spirulina cultivation
Strain selection
The major determinants in the selection of strains for commercial production are
growth rate
biochemical composition
 resistance to mechanical and physiological stress. A wide variety of species and strains of <i>Spirulina</i> have been screened by several people in various countries.
 Continuous mass production of a particular strain depends on its suitability and stability under prevailing conditions of the farm environment.

The strain selection that is very important as per spirulina cultivation is concerned. The major determinants is the selection of strain for commercial production are growth rate, biochemical composition, resistance to mechanical and physiological stress. A wide variety of species strains that is spirulina have been screened by several people by various countries. Continuous mass production of a particular strain depends on the suitability and stability under the prevailing condition of the farm environment.

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Now scale up of that spirulina cultivation. Culture is scale up from the mother culture that prepared in the lab the scale up follows roughly 5 is to 1 dilution ratio I told you that that

usually 5 to 10 percent we use here we use little bit higher it is goes as high as 20 percent through the successive volume of the culture that required culture in the production pond.

The second mode of operation culture expansion expansion to the enter volume of the production pond can be done from seedling in the concentration of 1 gram per litre. Through through careful manipulation of the nutrient concentration useful natural predators, it has it has been possible to maintain the mono algal culture even during the initial period of inoculation.

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The scaling up process in this stage where the contamination by other algae or bacteria poses the greatest problem because of the different dilute dilute nature of the spirulina inoculum. There there is a direct relationship between the density of spirulina in the culture and density of contaminants, contamination by green algae is a high when when the initial density of the inoculum is low because this is this is also very important factor that the inoculum volume plays very important role for this any fermentation process. If you inoculum is (())(28:02) this affect the fermentation process to a great extent. Conversely this amount of contamination decreases as the spirulina culture build up in density.

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So effect of density the light intensity plays very important role we find 30 to 40 klux find the normal optimum operation for spirulina. Outdoor algae culture exposed to two rhythms of dark and light regime.

The regime one is the relatively fast it is induced the mixing of the pond which results the turbulent flow of the culture and dictating the frequency of the life cycle, in this cycle the algal cell is shifted from the food solar radiation when located in upper culture surface and complete darkness when reaches the bottom culture usually in the depth of 12 to 15 centimetre. Other relatively slower regime is the range of solar irradiation during the day and the sun rise to sun set.

The two lifecycle light cycle impose a unique physiological regime on the adaption and acclimatization of outdoor algal cells to light.

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So the effect of light I do not like to tell in details because because as you know algae give some kind of pigment and it gives some kind of shading effect so that as as it grows very as the density increases light conversion efficiency that decreases to a great extent, so that give the self-shading effect that is the major problem with that so we cannot grow up to certain level So the light conversion efficiency automatically decreases.

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And pH also plays very important I told you the alkaline pH plays very important role in the algal that cultivation process pH adjusted by increasing carbon-di-oxide led by the addition of carbonate salts, the main area of losing carbon-di-oxide are exchange in the atmosphere and

precipitation of calcium carbonate loose lose to the part of the medium is not recycled back to the pond.

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Now harvesting I told you this is the we spent good amount of money for the harvesting process and this is this process if you want to look at the the economy of the process we shall have to make the harvesting process little bit cheaper. Then we observed that electro population technique is found little bit cheaper and it can be used effectively for the for the separation for the harvesting of the yeast cells.

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And drying you can see that how this that yeast cells the skinner and when you take the yeast cells it comes in the form of this solid mass it looks like this.

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And and then we shall have to filter it and after that we synthetic cloth and mesh of 30 to 50 microns is preferred for filtering medium. Supporting filtration cloth is fine net will accelerate somewhat the filtrating and protect the cloth against the rapturing. So this is this is the thing that we have.

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And then in the slurry obtained above 8 to 10 percent dry weight after filtration washed with the potable filtered water. This step is used for washing the excess salts from the biomass, it

amounts to 20 to 30 percent of dry weight. Final dewatering accomplished by pressing the biomass enclosed in a piece of filtrate cloth a strong cloth plus a strong cotton cloth either by hand or by any any kind of press. So this is the this is that that is used.



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And you see that after when you press it you get the algae in this dry form, it is this is this is not 100 percent dry but it is that we take out lot of moisture from it.

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And then this this we can we can put it in different forms you can see we can have we can put it some kind of mechanical device and through which we can make it in the form of thread you can see and then if you keep it in the sun you can dry it or you can keep it in the dryer it can dry very easily.

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And so sun dry is one of the most economical system for the small projects. In this, the concentrated biomass is spread thinner in the food grade polythene sheet and kept under the sunlight for 4 to 5 hours after drying the flakes is collected and stored clean and moisture less and opaque containers. The grinding process is done simply by mixer grinder.

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So this is like this this is the how the drying operation that is taken place in the different places.

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And and here I can show you the different forms the how the spirulina has been marketed. It forms in the marketed in the form of flakes and the form of powder, in the form of tablets, in the form of candies, in the form of capsules.

So in conclusion I want to tell that that spirulina is quite successful venture as per as per technology is concerned we find it has largely used throughout the world different companies are coming out for marketing this product and it is successfully they are producing in the both in mostly in the race open raceway pond and problem rise on couple of areas one is light penetration problem, another is light intensity, another is your harvesting problem.

So if we can contamination problem that is another problem that we use, growth characteristics of the organism that also plays very important role. Now if we can address this we can make the process much much cheaper, thank you very much.