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Cyanobacterial Cultivation on Dairy Effluent for Removal of Nutrient and its Biomass Production for Biofuel

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Cyanobacteria **grown on dairy effluent is a valuable source of low-budget production of lipid for biofuel and effluent treatment. Approximately 2-3 times greater amount of effluent generates for processing one liter of milk with high amount of organic & inorganic compound it can form to excess growth of algae, effect on biodiversity and decreased water quality level that may affect human and animal health. Technologies are available for removing these nutrients by chemical and physical means but requirement of energy and chemicals is greater, however, it increases cost of treatment.** ***Cyanobacteria*** **has capacity to utilize these inorganic nutrient such as N & P, it decrease the cost of removal pollutants and more easily collected biomass from treated effluent and become less expensive for biofuel production. The total quantity of lipid present in biomass ranged from 9% to 30%. The techniques for recovery of lipid from biomass by thermochemical liquefaction, pyrolysis and gasification and supercritical carbon dioxide extraction. Worldwide oil consumption is expected to 104 million barrels per day in 2030 from 86 million barrels per day in 2007, so we find the alternative with help of** ***Cyanobacterial*** **based biofuel production. Increase efficiency of** ***cyanobacteria*** **for lipid production with genetic engineering incorporation of specific gene which responsible for high lipid production.**

Key words: *Cyanobacteria,* Biofuel, Dairy effluent treatment, Genetic engineering.

Water pollution from dairy effluent has been major problem of environmental concern in countries around the world. This industry generated effluent volume is very large with its high nutrient characteristics, that's why this industry is more polluting and one of the most polluting not only in terms of the volume of effluent generated but also in terms of its characteristics. Approximately 2-3 times greater amount of effluent generates for processing one liter of milk (Vourch *et al.,* 2008).

High amount of organic and inorganic compound in dairy effluent can form to excess growth of algae, effect on biodiversity and decreased water quality level that may affect human and animal health. Technologies are available for removal these nutrients by chemical and physical means but requirement of energy and chemicals is greaterit increasing cost of treatment. For removing these pollutants use *cyanobacteria* have a capability to reduced level of it by biomass production and if these biomass collected it can be utilized for biofuel production. This process play a double role removing pollutants and produced economical products. Currently the difficulties in collecting the concentrating biomass but possible solution is *cyanobacteria* are grown on surface of dairy effluent as a biofilm then it is decrease the cost of removal pollutants and more easily collected of biomass from treated effluent and become less expensive for biofuel production in downstream processing.

In food industries and in some biotechnological applications *cyanobacteria* are mostly used as useful organism. (Thajuddin and

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Subramanian, 2005; Rastogi and Sinha, 2009). Photosynthetic algae contain material like vitamin, lipid, protein, and pigment (chromo and leuco pigment) and some secondary metabolite (Tan, 2007; Cardozo *et al.*, 2007).

Renewable energy source such as *cyanobacterial* biomass are becoming more important as substitute to fossil fuel. Biofuel production from *cyanobacteria* grown on dairy effluent has the possible to deal with three important public needs:

- Find a new energy sources
- Management of agricultural wastes to protect surroundings
- Decrease the risk of global anthropogenic greenhouse effect

Cyanobacteria are photosynthetic microorganism which produced sugar from sunlight, water and CO_2 and some other macromolecules such as lipid as a triacylglycerides (TAGs) are also obtained. The TAGs are the capable feedstock for biofuel production. *Cyanobacteria* have the ability to produce considerable amounts (20-50%) of triacylglycerols (TAGs) as a storage lipid under photo oxidative stress or other adverse environmental conditions. The development of dairy effluent treatment process an integrate *cyanobacteria* based method would simultaneously produce its biomass for biofuel and remove nutrients from dairy effluent. Biomass production and nutrient removal would be faster by addition of CO_2 from digester gas burning in a boiler or generator (Feffer, 2007).

Problem of dairy effluent

In a dairy the effluent is originated from product formation department and service sections. Leaked milk, wasted milk, skimmed milk and by products are the main sources for generation of effluent rich in nutrients, some are other source also generates effluent inform of whey from washing of milk can, equipment's, bottles and floor. Among these most difficult and high strength waste contain milk proteins, vitamins and some mineral salts is whey from cheese production and other dairy product formation.

Effluent volume from milk processing

The processing 4500-5000 litres of milk it generates effluent around volume is 20,000 litres. The average volume of effluent generated from milk processing is 2 to 3 times greater than actual volume of milk processed. (Bhadouria & Sai, 2010)

Characteristics of dairy effluent

The colour of effluent release from dairy is white and pH is slightly alkaline. In washing of milk canes, apparatus& other equipment's use cleanser that increase the pH and make it acidic by fermentation of milk sugar to lactic acid. Reductions of pH by this process casein precipitated out in effluent. Effluent contain high biochemical oxygen demand (BOD) as they have sufficient amount of carbohydrates. In high BOD level casein lead to decomposed and form heavy black sludge some strong acid like butyric acid which create odours in dairy effluent. With help of anaerobic process we can removes this strong odours compound (Wilkie, 2000).

Nutrients available in Dairy effluent

The dairy effluent including are quite rich in degradable organic matter and exert high oxygen demand. The dairy effluents are peculiar as compared to other industrial wastes because of relatively high concentrated effluent, particularly whey and butter washings. The processing of the one liter milk yield about 2-3 times greater effluent generates depending on the type of product manufactured. According to table 1 average composition of nutrient are present in milk and milk products. More than 90% of dairy waste consists of milk components (lactose, protein and fat) that are lost and flow into drains during processing describe in table 2.

In the untreated dairy effluent an average of biochemical oxygen demand (BOD) 0.8 to 2.5 kg/t. and chemical oxygen demand (COD) is 1.5 times higher from BOD level. 100-1000mg/l total suspended solids (TSS), 10-100 mg/l total dissolved solids (TDS) same concentration of phosphorus (10–100 mg/l) and lastly nitrogen concentration is present 6% from total BOD level. The cheese, cream, butter, and whey production is responsible for higher BOD level in dairy effluent. According to table 3 BOD & COD level present in milk and milk products. The organic components of milk present in effluent is equal to COD level like, 3kg COD = 1 kg milk fat, 1.13kg COD = 1kg lactose and 1.36kg COD = 1kg protein present in effluent. (Imam *et al.*, 2014)

The major nutrients in dairy effluent are nitrogen (N), phosphorous (P), potassium (K) and sulfur (S) given in table 4. Not all dairy effluent has the same nutrient content depends on the type of

effluent management system being operated.

In dairy effluent contain high amount of C source it decompose by microorganism like bacteria but bacteria has less capacity to remove nitrogen(N) and phosphorous(P) (Guzzon *et al*., 2008). Autotrophic c*yanobacteria* has capacity to utilize these inorganic nutrient such as N & P but heterotrophic bacteria carbon limited it only utilized carbon source (Stumm and Morgan, 1981). Common nitrogen removal methods such as bacterial nitrification/denitrification remove the majority of the nitrogen as a N_2 gas, while *cyanobacterial* treatment retains useful N compounds in the biomass. Though these benefits acceptable nutrient levels in the effluent cannot be achieved without sufficient production and harvesting of the *cyanobacterial* biomass. Unfortunately, use of algae in large-scale no any kind of technique has been developed as an inexpensive and simple (Uduman *et al.,* 2010).

Traditional methods for Dairy effluent treatments

Through biological treatment of effluent primarily decreasing BOD in processing of food. With aerobic treatment ponds & lagoons are used in land applications. Anaerobic treatment specially used as economical & viable while aerobic treatment used specially for greater BOD effluent (Green, 1979).

Aerobic technologies

- Trickling filter, activated sludge, rotating biological contactors, oxidation ditch, sequencing batch reactor.
- Anaerobic technologies:
- Continuous-flow stirred tank reactor, contact reactor, up flow sludge blanket, anaerobic filter (both up & down flow), Expanded or fluidized bed.
- In combination of both processes in single treatment system use in higher concentration stream anaerobic treatment is used for eliminating organic material while aerobic treatment for lower concentration streams effluent but these treatments has some difficulties to remove inorganic materials are as follow,
- Elimination of N and P
- Biological degradation of fats, oils and grease
- Biological growth separation, sludge clearance, bubbling and layer creation

Developing Technologies

Available technologies has a challenge to give effective treatment of wastewater more than 2% of suspended solids in the wastewater. For these challenges some new technologies are developed to increase effectiveness such as, Thermophilic Processing, Solvent Extraction, Bioadditives, Biodenitrification, Hydrothermal Processing and Membrane technology.

Comparison of *cyanobacterial* treatment with physical and chemical processes *cyanobacterial* treatment has great ability to removal of nutrient in low cost and provide more benefits in economy with recycling & renewable energy (Oswald, 2003).

Cyanobacteria

Autotrophic *cyanobacteria* has capacity to utilize inorganic nutrient such as N & P. *Cyanobacteria* are one of the ancient photosynthetic organisms on earth. They are autotrophic organisms which absence of internal organelles, a separate nucleus and the histone proteins associated with eukaryotic chromosomes. Photosynthesis occurs directly done in the cytoplasm of the cell. Photosynthetic lamellae have some pigments like phucoerythrin & phycicyanin which give blue green color to *cyanobacteria* and it also have chlorophyll a. (Owuor *et al.,* 2007).

Temperature and *Cyanobacteria*

Cyanobacteria can grow in an extensive range of temperatures but really start to grow when water temperatures exceed 20°C (Owuor *et al.,* 2007). Lower temperatures inhibit the growth of *cyanobacteria* even in the presence of high concentrations of dissolved nitrogen (e.g. NO_3^-) and phosphorus compounds (e.g. PO_4^{3-}) in surface water which present high amount in dairy effluent. *Cyanobacteria* frequently dominate eutrophic water systems during the high temperature period of the year (Davis *et al.,* 2009). Low temperature one of the limitation of *cyanobacterial* growth.

Nutrients and *Cyanobacteria*

Nitrogen and Phosphorus are the two most important nutrients for *cyanobacterial* growth. Unlike other algal and diatom species, *cyanobacteria* have the capability to fix nitrogen and store phosphorus for future use which give them a good advantage to grow in phosphorus-rich and nitrogen contain water systems. Dairy effluent good nutrient source for *cyanobacterial* growth.

***Cyanobacterial* reproduction**

Reproduction in *cyanobacteria* occurs through a different methods, such as binary fission, budding or fragmentation. These various methods of reproduction cause *cyanobacteria* to act in many different forms such as coverings, oily masses, strings, filaments and branched filaments. Binary fission includes the replication of DNA. Development of smaller cells from bigger cells called budding while in fragmentation microorganism breaks in small fragments and each fragments developed into new organism. The form of *cyanobacteria* that will grow is determined by the wavelength of photosynthetic light that is available. Nutrients such as phosphates and iron are also important in *cyanobacterial* growth and reproduction which available in dairy waste water in sufficient quantity. (Owuor *et al*., 2007).

***Cyanobacterial* organization**

Cyanobacteria have the ability to form many types of organizations, depending on environmental factors such as warm temperatures and the availability of phosphorous and nitrogen. *Cyanobacteria* can grow as epilithic forms which grow on rocks and stones, grow on plants in the water called epiphytic forms while grow on the bottom deposit of dams called epipelic forms.

***Cyanobacteria* different organizations form are as following (Owuor *et al*., 2007):**

- Slime like material surrounded in single cell
- Colonies of flattened sheets
- Colonies of cubed or rounded balls
- Colonies with elongated filaments

Filamentous colonies of *cyanobacteria* occur in the following three cell types (Owuor *et al*., 2007);

- Photosynthetic growing cells are formed in good conditions
- Climate-resistant spores may form under environment tough condition
- Thick-walled heterocyst cells

Benefits of *Cyanobacteria*

The plant-like organisms employ photosynthesis to convert sunlight and CO_2 into energy, so efficiently they can dual their mass several times a day and have the potential to produce significantly higher lipid than corn and soybeans in one acre given in table 5. The amount of lipid produced by *cyanobacterial* biomass range from 5% to 51% is depending on type of *cyanobacterial* species, nutrient availability and growing condition. *Cyanobacterial* grow well on a nutrients of wastewater and carbon dioxide a greenhouse gas. Wastewater is attractive because it has the high nutrients value for *cyanobacteria* need to consume especially phosphates and nitrates but wastewater only does not provide a balanced nutrition. Most wastewater has too much N and P but not enough carbon. *Cyanobacteria* cannot adapt only in presence of N and P, carbon dioxide must be added. CO_2 is generated from several wastewater treatment plants when sludge is burned or treated in an absence of oxygen digester to produce biogas (Greer, 2009).

Using *cyanobacteria* produced biofuel is more economically feasible or provide renewable energy. Dual-use of *cyanobacterial* cultivation for dairy effluent treatment with dual-role biofuel production and wastewater treatment.

Requirement of Biofuel as an alternative source

According to the Energy Information Administration (2010), worldwide energy demand is expected about 49% increase in the next 25 years. Worldwide oil consumption is expected to 104 million barrels per day in 2030 from 86 million barrels per day in 2007. The world's total verified oil reserves are estimated at 1.293 trillion barrels according to the Oil & Gas Journal (2005). Under these growth assumptions about half of the world's total oil reserves would be exhausted by 2030. As these oil reserves counsellor by alternative liquid fuels like biodiesel and bio-ethanol are becoming more and more essential as substitute energy sources to oil. In 2004 world produced over 33 billion liters of these biofuels. As a substituted of fossil fuel biofuel is fasted growing with more than 32% production per year and production assumption is 12 billion liter at 2010 (Lim and Teong, 2010). Among the many oil sources are available for biodiesel production, such as soybean, rapeseed, sunflower and palm oil, microalgae promise the highest yield of oil per kilogram of biomass and have more capability faster growing than crops (Chisti, 2008).

Isolation of *Cyanobacteria*

Isolation and purification method of *Cyanobacteria* according to Ferris and Hirsch (1991).

- Collect five liter Dairy effluent samples from site.
- First shake the sample and allow to sediment suspend this process do three time for removed suspend and then add 100 ml sterile distilled water and filtered by Millipore filters.

- Then filter were place on BG-11 (Blue-Green) medium containing petri plate. For 15 days incubate it with microscopically examined for the growth of organism.
- During/After incubation different species were grow in medium. Aseptically each species were picked and sub-cultured in 500 ml liquid medium for growth of organism and incubated under continuous lighting (2000 lux) 14:10 h light: dark system at 28±2°C.
- Using of BG-11 medium without N source carried out isolation of *calothrix fusca* & *scytonema bohneri* and also maintained by same medium then analysis of harvested cultures after 30 days by biochemical constituents.

***Cyanobacterial* Biodiversity in dairy effluent**

Total 27 species of *cyanobacteria* were found in dairy effluent. Out of them, *Oscillatoria subbrevis* was dominant as compared to 12 other species of *Oscillatoria*, some common genera which found in dairy effluent are given in table 6 and common species in table 7.

Isolation and maintained c*yanobacteria* two medium are used such as BG 11 (Blue Green) & Bold Basal medium.

Additional requirement for better treatment

The combination of biofuel production and wastewater treatment with CO_2 supplementation as prior suggested by Oswald and Golueke (1960). In particular utilization of wastewater nutrients by *cyanobacterial* biomass were collected and considered as a potentially practical and economical approaches to biofuel production and *cyanobacterial* based treatment of dairy has been studied (Craggs *et al.* 2004, Kebede-Westhead *et al.* 2006, Mulbry *et al.* 2008). Lipid content for pure cultures of *cyanobacteria* have been reported to range from 1%-85% and the lipids exhibit varying carbon chain lengths, degrees of unsaturation, and polarity given in table 8 (Chisti, 2007). However the amount of lipid produced by different culture of *cyanobacteria* is more important, such as dairy effluent tank. Environmental conditions, methods of culturing and growth phase highly affect the fatty acid profile of lipid and growth of biomass, Thompson (1996). In some species increase the productivity of lipid by decreasing N source Spoehr and Milner (1949). But decreasing of N source affect the growth of biomass and ultimately decrease overall amount of lipid. Benemann and Tillett (1991) studied this difficulty but increasing amount of lipid productivity is remain an unresolved problem.

Table 1. Average composition of Nutrient in the milk and milk products (Dr. A. S. Kolhe, S. R. Ingale, 2009)

Sr. No.	Dairy Product	FAT (%)	Protein (%)	Carbohydrate (%)	Total Solids(%)	Chloride mg	Calcium mg
1	Flavored milk	4.5	3.8	11.00	20.00	100.00	118.00
2	Skimmed Milk						
2.1	Buffalo	0.1	4.2	5.4	10.5	39.00	150.00
2.2	Cow	0.1	3.6	5.0	9.4	35.00	130.00
3	Butter 2% salt	81.0	0.6	0.4	84.5	780.0	20.0
4	Ghee	99.5	0.1	0.0	99.7	896.0	—
5	Ice-Cream	10.0	5.0	17.6	23.4	180.0	150.00

Table 2. Concentration of organic components in dairy effluent (Ashish Tikariha, and Omprakash Sahu, 2014)

Sr. No.	Name of components	Concentration in mg/L
1	Protein	13.78-72.12
2	Carbohydrates	0.1007-0.2958
3	Fat	0.01-0.06

Table 3. BOD & COD according to milk and milk products

Product	BOD5 mg/l	COD mg/l
Whole milk	114,000	183,000
Skim milk	90,000	147,000
Butter milk	61,000	134,000
Cream	400,000	750,000
Evaporated milk	271,000	378,000
Whey	42,000	65,000

Recently a few species of *cyanobacteria* have been studied for their ability to convert solar energy to chemical energy has been found to be the most efficient for biofuel production among all living organisms. The capacity of solar energy conversion in corn and sugarcane is 1–2% and for eukaryotic algae it is around 5% while for *cyanobacteria* it is 10%. *Synechococcus elongatus* this transgenic strain produced isobutyraldehyde which could be recovered and converted to butanol.

Basic steps for biofuel production from biomass cultivated on dairy effluent

There are five major steps in the production of biodiesel from *cyanobateria* biomass derived oil:

- Growing of *cyanobacteria* biomass.
- Collecting of *cyanobacteria* biomass.
- Extraction of oils from the *cyanobacterial* biomass.
- Produced Biodiesel from oil via transesterification.
- Last step is separation of biodiesel from crude then purified the biodiesel.

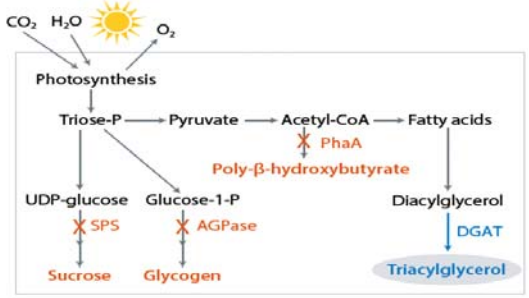

Fig. 1. NREL Modified pathway

Extraction of oils from *cyanobacteria*

There are many processes in literature to extract oils from *cyanobacteria*. Including gasification thermochemical liquefaction, pyrolysis, and supercritical CO_2 extraction. Liquefaction is the more preferred than thermochemical process to produce biofuels from *cyanobacteria* because of requirements of energy is lower compared to pyrolysis and gasification. Thermochemical liquefaction occurs between 200-300°C while pyrolysis and gasification occur at 500°C and 2000°C respectively. Aresta *et al.* (2005) compared the extraction of *cyanobacterial* oils through thermochemical liquefaction and supercritical CO_2 extraction and found that thermochemical liquefaction was more efficient from a quantitative point of view. Barnard, 2009 found that thermochemical liquefaction was a preferred option to extract oil from microalgae. He found that the optimum process conditions in the liquefaction reactor of 300°C, 15000 kPa, and 3% dry weight microalgae with the remainder being water solvent produced the maximum oil 15.60% in 30 minutes. After liquefaction Barnard added 20% chloroform solvent and agitated the mixture at 750

Table 4. Ratio of the nutrients: N, P, K and S in dairy effluent(Guzzon *et al.*, 2008)

	Typical ratios of nutrients in %			
	N	P	K	S
Dairy Effluent	9	1.5	11	1
Pasture nutrient requirements	5 (fertilizer N only)	1.6	2.6	1
Crops	8	2	8	1

Table 5. Production capacity of biofuel from different sources

Source	Gals/acre	L/hector
Soybean	50	92
Corn	250	458
Sugarcane	450	825
Oil palm	650	1192
Algae	2000	3668
Joule technologies	15,000	27,512

Table 6. The Common Genera (K. N. Pathade, 2012)

Filamentous		Unicellular And Colonial
Oscillatoria	Ananbaena	Microcystis
Lyngbya	Aphanizomenon	Synechococcus
Spirulina	Calothrix	Anacystis
Nostoc	Rivularia	Gloeocapsa
Cylindrospermopsis	Gleoetrichia	Agmenellum(syn. Merismopedia)
Planktothrix		

rpm for ten minutes to recover oils from the water phase. Barnard then used vacuum distillation at 70°C to isolate the micro-algal oils successfully from the chloroform solvent.

Biofuel production from *cyanobacterial* lipids

There are mainly 4 methods to produced biodiesel from *cyanobacteria*:

- Direct use
- Blending
- High temperature decomposition (pyrolysis)
- Transesterification (Ma and Hanna,1999).

Among these the best method is transesterification in this method biodiesel is directly utilized or blending with diesel (Peterson *et al*., 1991; Zhang *et al*., 2003). Miao and Wu (2006) produced biodiesel in four hours of reaction time through the acidic transesterification of biomass oil. They found that the best process at a 30°C temperature 56:1 methanol to oil ratio reagent quantity combination with 100% sulphuric acid (based on oil weight). They suggested that their process was feasible and effective. For the transesterification of algal oil alkali reagent not suitable and he recommended that it was may be due to higher amount of acid in algal oil.

Genetic engineering for more oil production

During photosynthesis conversion of CO_2 to sugars like glycogen and other carbohydrates which is energy source stored in *cyanobacterial* cell. Discovered the actual process of photosynthesis and do some changes in pathway by National Renewable Energy Laboratory (NREL) scientist for production of lipid and other important organic acid by photosynthesis. With help of research could create new source of biofuels from *cyanobacterial* lipid. *Cyanobacterial* lipid have potentiality to convert into fuel. Scientists chose a fully sequenced genome species of c*yanobacteria (Cyanobacterium synechocystis* 6803) and scientist have an all genetic tools to modify any gene to check the effect of those modification. The modification in genetic level mostly for increase the production of oil by *cyanobacteria* is challenge because in natural form *cyanobacteria* does not produced much more lipid.

In 2009 scientist of NREL start to genetic manipulate of *cyanobacterial* photosynthetic pathway for production of higher amount of lipid quantity. The work started with three initial experimental goal.

- To evaluate the flow of carbon within bacteria for blocking glycogen synthesis.
- Incorporation of gene for switch on the production of triacylglycerol with manipulation for physiological use and improve the TAG quantity

Table 7. The Common Species (K. N. Pathade, 2012)

Phormidium anomala	Lyngbya aestuarii
P. incrustatum	L. lagerheimii
P. submembranaceum	Microcystis aeuroginosa
P. tenue	M. flosa-que
P. papyraceum	Plectonema nostocorum
P. ambiguum	Aphanocapsa pulchra
P. tomasinianum	Synechococcus

Table 8. Fatty acid composition (in %) of seven species of *Cyanobacteria* (K. R. Rajeshwari & M. Rajashekhar, 2013)

		Sulfur spring water			Petrochemical Effluent	Sewage water	Dairy effluent
Fatty acidProfile	*Lyngbya limnetica*	*Calothrix Fusca*	*Scytonema bonheri*	*Gloeocapsa Livida*	*Oscillatoria calcuttensis*	*Oscillatoria acuminata*	*Oscillatoria Foreaui*
C8:0Caprylic acid	3.36±0.7	10.45±1.2	9.58±0.5	8.10±0.1	8.13±2.3	7.59±0.8	7.45±0.3
C10:0Capric acid	1.99±1.2	6.85±0.6	5.99±0.3	5.86±0.5	5.11±0.5	4.66±1.9	5.79±0.6
C12:0Lauric acid	17.17±0.9	41.39±0.2	41.40±0.4	45.30±0.6	35.29±0.3	33.33±0.3	44.53±0.6
C14:0Myristic acid	5.32±2.1	11.92±0.4	10.78±0.7	16.77±0.9	10.42±1.2	10.58±0.7	10.65±0.8
C16:0Palmitic acid	6.09±0.2	10.04±1.3	8.33±0.3	4.69±2.0	7.46±0.5	8.35±0.2	7.17±1.1
C16:1Palmitoleic acid	2.48±0.5	Nil	Nil	Nil	0.27±1.5	Nil	Nil
C18:0Stearic acid	1.60±1.5	1.65±0.8	1.88±0.5	Nil	1.64±1.7	Nil	1.66±0.5
C18:1Oleic acid	3.10±1.1	2.47±0.7	2.09±1.9	2.91±1.0	3.72±0.7	3.89±2.2	1.68±2.3
Linoleic acid	1.56±0.5	1.14±0.4	Nil	Nil	2.18±0.3		1.98±1.5
C18:3Linolenic acid	Nil	Nil	Nil	Nil	Nil	Nil	2.28±0.6

and also add biodiesel precursor.

NREL scientist select the production of glycogen from glucose 1- phosphate pathway of *cyanobacteria* model *synechocystis* 6803 and block the gene which responsible for production of the AGPase enzyme.

Creating a New Oil Synthesis Pathway

The scientist team firstly focus on *synechocystis* genes which responsible for naturally synthesis of lipids and find out the pathway of lipid synthesis represented in Fig. 1. They try to addition of enzyme such as diacylglycerol acyltransferase would permit to TAG production by organism. The group of scientist working for to prepared a good-characterized gene sequence for the enzyme DGAT and they design new gene for *synechocystis* then new gene transferred into organism. Then presence of DGAT in organism it looked like mutant strain.

In 2012, confirmed by the NREL scientist team after the testing of DNA and protein of DGAT present in mutant strain. After this they give confirmation of TAG synthesis pathway predicted and producing TAG in mutant strain compared with wild type *cyanobacteria* doesn't have TAG pathway. However, the production of TAG level is low it produced less than 1% of the cell biomass found by NREL team but is was establishment of starting point of *cyanobacterial* based biofuel production with economical point of view.

CONCLUSION

Cyanobacteria have a capacity of utilization of nitrogen and phosphorus and decreasing concentrations in dairy effluent through biomass production and from collected biomass suggestion to added extra benefit as a source of lipid for the production of biofuels and biological products with genetic modification, we can increase the quality and quantity of product. The integration of *cyanobacterial*- based biofuel and biological products production with removal of pollutants by effluent treatment advantages for industries.

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