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The IJEHS is an official publication of Save The Environment (STE). It publishes peer reviewed quarterly, original articles (Research paper, Review articles, Short Communication, Case studies, etc.) related to all fields of Environment and Health Sciences. It disseminates the scientific research and recent innovations.

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International Journal of Environment and Health Sciences

From The Editor's Desk…

Advent of modern technologies is leading to persistent urbanization and capitalism which although favorable to mankind, have a plethora of environmental hazards accompany them. Air pollution, water contamination, greenhouse gas emissions, etc. are manmade tribulations that are challenging the availability of clean air and pure water. Consequently, health perils linked to environmental risk factors are snowballing at an alarming rate. In this outlook, the necessity for formation of regulatory bodies to propagate awareness for environmental sustainability is more now than ever before.

With this perspective, the International Journal of Environment and Health Sciences (IJEHS) proposes to provide a reliable platform to discuss technologies and strategies for management of aforesaid environmental matters. IJEHS has been launched as a peer-reviewed quarterly journal that will be quintessential to academicians, industry professionals and researchers who are actively engaged in the areas of environmental issues and related health effects. We are pleased to inform that ISSN for IJEHS is now available as 2582-5283.

We invite original research articles, short communications and critical reviews directed towards an academic, clinical and industrial audience. The first section of the journal focuses on burning environmental issues like pollutants and their fate, waste management, resource conservation, remediation technologies, etc. The second section includes all topics relevant to physiological impact of environmental risk factors and application of alternative medicinal approaches as remedial measures. Detailed scope can be found in the home page of the journal (www.stenvironment.org). Notes on development of any novel and validated strategy or tool to address environmental challenges are welcome. Discussion on proceedings of conferences conducted around an environmental theme will also be considered. All submissions will be meticulously scrutinized by pioneers in the field to ensure publication of only articles of high quality and relevance. Authors are requested to take special precautions to avert plagiarism and redundancy.

It is high time that we realize the gravity of circumstances and take potent steps to undo the adversities already triggered. The time is now and the place is here. With this, I wish all our readers a Very Happy New Year, 2020 and I hope our audience and patrons shall come together in this effort to promulgate their part in resurrecting our valuable environment.

Dr. Kshipra Misra
Editor-in-Chief, IJEHS

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INDIANS WILL REMAIN RELATIVELY SAFER FROM COVID-19 DUE TO GOVERNMENT MEASURES AND FOOD HABITS IN INDIA: WILL NOT ENTER STAGE-3 & STAGE-4

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Abstract
A unique genetic makeup, rich traditional uses of Indian medicinal plants and traditional foods by different communities in India will not let the corona virus multiply in the body and body's immune system will kill the viruses. This report has been intended to present impact of COVID-19 in India and health implications on Indian populations. India will remain safer relatively from menace of COVID-19 largely due to reasons described in text.

Keywords
COVID-19, Indian traditional medicine, immunity

1. Swift and robust measures by Central and state governments:

Indian government's swift action started as early as on January 17, 2020. WHO said on 24 January, that COVID-19 won't be an emergency, which turned out to be major failure of WHO expertise.

But Indian government visualizing the threat acted using its wisdom and took drastic measures to control the virus with multipronged strategies. Modi government wasted no time and started screening of passengers, potential carriers of coronavirus, at all international ports in India.

The screening of passengers from China had begun on January 17 and was later expanded to cover another 13 affected countries but with the detection of 39 cases, the government has escalated the preventive measures at all airports.Till 14th Feb, only passengers arriving from four countries -- China, Hong Kong, Thailand and Singapore were being screened for possible exposure to the respiratory virus at 21 identified airports in India. And later, applied to all the flights arriving from all the countries. All domestic airlines were barred from flying from 24 March and all international flights were suspended from 22nd March. By 4 March, Government of India had screened as many as 6, 11, 167 passengers and by the end of March, Government screened a whopping 15, 24, 266 number of passengers at all Indian ports of entry.

In an unprecedented move, the railways also announced the suspension of all its passenger services from March 22 midnight amid the coronavirus pandemic. Movement of around 500 million people daily by railways was stopped where several hundred thousand people could have been infected through contacts. Railway stations and trains are among the most crowded places where chances of infection through human contact were very high. Country announced total lockdown for 21 days from midnight of 25 March. Thus, vast number of 135 million of dynamically moving people who could be potential spreaders and infected with coronavirus were brought to sudden halt. The chances of infected persons acting like asymptomatic excerptors and like a chain reaction were put on immediate brake. All above measures worked like graphite rods to retard thermal neutrons, which were responsible for nuclear chain reaction in a nuclear reactor. Had these measures not been adopted, the coronavirus would have exploded like a nuclear bomb in the second most populated country in world, surpassing cases manifolds compared to USA, Italy, Spain and other countries. It has saved and would further save millions of lives with stringent steps taken at a lightning speed. Prime Minister | Mr. Narendra Modi deserves Nobel Price for peace for saving millions of lives through his great political acumen and decisiveness ability. His measures will pull millions of families out of danger who would have had otherwise drowned in tears due to losing their loved ones. His measures will help break the cycle of corona virus one hand, on the other hand, all confirmed cases of COVID-19 would surface and taken by robust and alert medical team for treatment. Thus, halting all railways, all flights, state buses and total lockdown for 21 days would prove highly beneficial in controlling COVID-19.

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Apart from the center, state governments like Delhi, Punjab, Uttar Pradesh, Haryana and others became vigilant equally and took several measures to control dreaded spread of COVID-19. All states wholly supported measures of central government and showed great practical approach at the time of national crisis.

1. Herd Immunity

The genetic constitution of Indian sub-continent population is unique. It has evolved in such geographical conditions that grants population more robust immune system. It is this immunity that kills any foreign invasion in the body including viruses and novel viruses. The Indian population is exposed to varied antigens including various strains of flu viruses, herpes simplex viruses, hepatitis viruses in atmosphere and makes B-cells to do class switching to produce more varied immunoglobulins and initiate cell mediated immunity. Immunity developed against different viruses also to some extent protected people from COVID-19. There are many behavioral similarities between Herpes simplex virus strains and COVID-19. Both are highly contagious; both have route of infection through eyes, nose and mouth; both are asymptomatic excretors; both attack immune-compromised population (especially elderly people) and both cause respiratory infections leading to pneumonia disease. Exposed to such varied antigens, there is tremendous generation of diversity of antibodies. It is like natural vaccination of the population. The overall effect is such that it makes body prepared for even novel types of antigens including novel corona virus disease COVID-19. Thus, studies on genetic makeup of Indian population have proven that it is favoring strength of immune system. It should not be confused with immunological disorders in Indian population, which does not warrant mention here.

According to Indian Genome Variation Consortium (IGVC) in 2008, “while it wasn't surprising to find similarities in the genetic profiles of people living in opposite corners of the country, considering the tremendous number of migrations throughout Indian history, most groups maintain their genetic uniqueness, unlike populations in the United States or Europe”. Scientists here have completed a study mapping the genes of various ethnic groups in India, the largest such study on any population anywhere in the world that shows which groups are susceptible to which diseases and responsive to which medicines. According to this study, people in parts of Punjab, Haryana and Kashmir have a natural immunity to HIV/AIDS. Council of Scientific and Industrial Research, the Indian Genome Variation Consortium (IGVC) including at least 150 scientists has mapped nearly 1,871 human genomes, sourced from 55 endogamous (pertaining to a community, clan or tribe) populations.

In another study published in Nature India, August 2008, an Indo-US team of researchers has found that Indians, compared to other world populations, carry more of a kind of natural killer cells that can detect and terminate infections at an early stage. Research suggests that Indians acquired the activating KIR (killer cell immunoglobulin-like receptors) genes as a result of natural selection to survive the environmental challenges during their pre-historic coastal migrations from Africa. White blood cells, or leukocytes, are cells of the immune system defending the body against disease by identifying and killing pathogens and tumor cells. The main types of WBC are T lymphocytes, B lymphocytes, macrophages, neutrophils and natural killer cells. The defense in the first week of infection is mediated primarily by macrophages, neutrophils and natural killer cells. In most cases, these cells do a great job of keeping people healthy and preventing infections. If the infection is not contained by the first week, the T and B lymphocytes will initiate more specific and strong defense. White blood cells, or leukocytes, are cells of the immune system defending the body against disease by identifying and killing pathogens and tumor cells. The main types of WBC are T lymphocytes, B lymphocytes, macrophages, neutrophils and natural killer cells. The defense in the first week of infection is mediated primarily by macrophages, neutrophils and natural killer cells. In most cases, these cells do a great job of keeping people healthy and preventing infections. If the infection is not contained by the first week, the T and B lymphocytes will initiate more specific and strong defense (Nature India, August 2008).

According to one more evidence, people of African ancestry generally show stronger immune responses than Europeans do. The discovery suggests that European populations have been selected to display reduced immune responses since our ancestors first made their way out of Africa. Intriguingly, the immune systems of Europeans were partly shaped by the introduction of new genetic variants through interbreeding between some of our early European ancestors and Neanderthals. Two studies reported in Cell on October 20, 2016 show that those differences in disease susceptibility can be traced in large part to differences at the genetic level directing the way the immune systems of people with European and African ancestry are put together (Quach *et al*., 2016).

2. Indian Food Habits

Indian population is largely vegetarian and user of traditional foods in their diet. India is agriculture based country and user of fresh vegetables mostly. Very less number of population is dependenton processed and frozen products. The processed and frozen food does not favor body's immunity whereas fresh vegetables affects immune system more favorably. It suppresses pro-inflammatory cytokines like TNF-alpha and Interleukin-1 (IL-1) and promotes anti-inflammatory cytokines like interferons and IL-4. Our diets rich in anti-oxidants lignans, flavonoids, polyphenols, triterpenes and essential fatty acids like Alpha Linolenic Acid (ALA) etc. reduce oxidative stress in the body and acts on immune system to activate it favorably. For example, lignans and ALA act together on membrane phospholipids and alters in such a way that viruses cannot penetrate through the membrane including membrane of respiratory tract. As COVID-19 is an example of zoonotic transmission it might be attacking more on people whose diet is rich in meat and especially beef and

pork and do not have much anti-oxidants like lignans, flavonoids, polyphenols, triterpenes etc. As basis of any infection is host-parasite relationship, people dependent more on meat diets and with less plants based anti-oxidants may attract more infection. According to a research published by Sarkar *et al*. (2015), traditional Indian foods have been prepared for many years and preparation varies across the country. Traditional wisdom about processing of food, its preservation techniques, and their therapeutic effects have been established for many generations in India. Food systems can deliver numerous biological functions through dietary components in the human body. Indian traditional foods are also recognized as functional foods because of the presence of functional components such as body-healing chemicals, antioxidants, dietary fibers and probiotics. These functional molecules help in weight management, and blood sugar level balance and support immunity of the body. The functional properties of foods are further enhanced by processing techniques such as sprouting, malting, and fermentation (Hotz & Gibson, 2007). Many traditional foods are good sources of flavonoids, minerals, vitamins, carotenoids, electrolytes and other bioactive compounds that have impact on human health. (Sarkar *et al*., 2015).

3. Traditional Practices of Herbal medicines

About 80% of Indian population uses traditional medicines routinely for ailments like flu, cough, fever, headaches, pain, inflammation, diarrhea etc. Traditional medicines include ginger, turmeric, clove, long pepper, cinnamon, nut meg, holy basil, black peppers, brahmi, shankhpushpi, kali museli, safed museli, mentha, ajwain, rai, anant mool, Giloe and many more. Such traditional medicines influence immune system favorably and fight with infections. As mentioned above, these traditional medicines are rich in anti-oxidant properties and germicidal. Many traditional food items like flaxseeds, sesame seeds, Arabica gum etc. are made in to different kinds of preparations and eaten routinely in many parts of the country. People in different parts of country have evolved their own food habits those promote good health and immunity. Thus, a rich traditional use of Indian medicinal plants by different communities will not let the coronavirus multiply in the body and body's immune system will kill the viruses. Uses of medicinal compounds like Loban, Kapur, Gugal, Lawang, Dhup, Til etc. in Puja-Havan has been listed in Ayurveda as respiratory disinfectants. It acts as sanitizer for respiratory tract from nostrils to lungs. There may be nationwide massive havan in different communities to stop corona infecting respiratory tract. It is being reported now that in Wuhan (China), all COVID-19 infected patients were treated with Traditional Chinese Medicines (TCM) along with modern therapy and in 90% cases of infected people in China, TCM was practiced. One must be surprised that in China 83,059 cases were confirmed and out of these highly significant numbers 76,052 cases were recovered and 3,305 deaths were recorded. It is astonishing that 91.56 percentage of people recovered!! Either China already has vaccine against the COVID-19 or the TCM must have played the magic. Thus, our country should also leave no stones unturned to try Indian Traditional Medicines for preventive measures. There are many such herbs those if used as preventive medicines, could bring down the numbers of casualties. Tibet and Japan are least affected from COVID-19 as both are highly dependent on traditional herbs which possess great healing power and highly rich in anti-oxidant compounds and wonderful immunity booster. Use of plants as a source of medicine has been an ancient practice and is an important component of the healthcare system in India. In India, about 80 percent of Indian population tried the traditional Ayurvedic system of medicine many times. Most healers/practitioners of the traditional systems of medicine prepare formulations by their own recipes and dispense to the patients. In the Western countries, approximately 40 % of people are using the herbal medicine for the treatment of various diseases. (Pandey *et al*., 2013).

Thus, keeping the current trend when total cases of corona infected people in the country are about 1000, recovered cases 124 and total deaths 25, the coronavirus has no chance to enter in to community stage-3 and epidemic stage-4 and the country would be safer from killer COVID-19. In India, total confirmed cases would even never touch the number of deaths in some countries like US, Spain and France. Italy's figure of deaths is too high to reach in India. Barring a few ignorance by public and group of people, those ignored government advisories, and keeping in mind current trend, the expected confirmed cases would range between 5000 to 7000 cases and deaths should not go beyond 7% of confirmed cases when country will finally be free from COVID-19 in a couple of months.

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ENVIRONMENTAL RISK FACTORS OF LUNG CANCER

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Abstract
Lung cancer previously known as a "smoker's disease" now has many other factors known which show synergistic effects in its development. Increasing incidence in non-smokers suggests the potential role of environmental and occupational factors as a risk for the development of this disease. The review highlights current knowledge of the environmental triggers and their role in lung cancer. Interventions taken to address this deadly disease have also been discussed.

Keywords
Lung cancer, environmental determinants, mutations, occupational factors, Interventions.

Introduction

Lung cancer is the leading cause of death in both men and women worldwide (Siegel *et al*., 2020). It is the fourth common cancer after the colon, breast, and pancreatic cancers (Zappa *et al*., 2016). Over half of patients diagnosed with lung cancer die within one year of diagnosis. World Health Organization (WHO) had classified lung cancer in two main subtypes: small cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC), accounting for 15% and 85% of all lung cancer, respectively (Inamura *et al*., 2017). It is an asymptomatic disease; initial symptoms are similar to flu. By the time it is correctly diagnosed the patient may be in the advanced stage of cancer. Late-stage diagnosis makes the applicability of surgery and radiotherapy less favourable for the treatment and hence, chemotherapeutic regimens are preferred. On the other hand, for SCLC, which is already metastatic at early stages, no effective targeted therapy has been identified as yet.

To develop an effective treatment regimen, it is important to understand the causative factors and their role in its development. Lung cancer has been primarily linked to tobacco smoking however, its increasing incidence in non-smokers and children has been reported. These can be attributed to environmental and occupational exposure to various kinds of hazardous substances. Because of increasing industrialization and environmental pollution around the world, the etiological factors of lung cancer have become more multi-fold.

Risk factors

SCLC is composed of much smaller cells which rapidly metastasizes to other organs much faster than NSCLC. It can be fatal within a few weeks if untreated, in contrast to most cases of NSCLC with metastasis (Johnson, 1993). The main aetiological factor in lung carcinogenesis is tobacco consumption (Devesa *et al*., 2005). In 80% of deaths due to lung cancer, smoking is the key reason and almost all patients with SCLC are current or former heavy smokers (Furrukh *et al*., 2013).

Patients who are not having a history of smoking have also been detected for NSCLC. A meta-analysis study observed a positive association between environmental exposure to air pollutant and lung cancer in 8 out of 14 studies with a relative risk range 1.14- 5.2 (Whitrow *et al*., 2003). Relative risk range indicates that the risk of developing lung cancer is 1.14-5.2 times more than the average risk when subjected to environmental exposure. It is calculated by dividing the probability of an event occurring for group 1 divided by the probability of an event occurring for group 2. Several studies reported factors like air pollution, exposure to radon and asbestos as also risk factors for lung cancer (Speizer 1983; Bradford,1965; Morabia *et al*., 1992). Other major determinants such as genetics, occupation, poor diet also contribute (Osada et al.,2002). Changes in genes (oncogenes, suppressor gene) may also lead to lung cancer (Kanwal *et al*., 2016). SCLC female patients have shown parental history (Planchard *et al*., 2015). EGFR gene change is seen more often with non-smokers having adenocarcinoma of the lung in young non-smoking Asian women, but the excess EGFR protein has also been seen in more than 60% of metastatic NSCLCs (Varella-Garcia M.,2010). RB1 tumor suppressor gene is thought to be important in the development of SCLC whereas, acquired changes in p16 tumor suppressor gene and

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the K-RAS oncogene, are thought to be important in the development of NSCLC. Changes in the TP53 tumor suppression gene and chromosome 3 can be seen in both NSCLC and SCLC (Mogi *et al*., 2011; Naylor *et al*.,1987).

Environmental determinants

Chemical exposures

A study on exposure to arsenic, butadiene, cadmium, chromium, formaldehyde, and nickel has shown their role in lung cancer (Luo *et al*., 2011). Other chemicals like aflatoxin B1, dioxin and chlorophenols (CPs) also have a carcinogenic effect on lung. Aflatoxin B1 is a common contaminant in a variety of foods including peanuts, cottonseed meal, corn, and other grains; as well as animal feeds that can cause primary lung cancer (Cui *et al*., 2015; Dvorackova *et al*.,1981). CPs and dioxin compounds are pesticides that have been associated with this disease as proven experimentally (Walker *et al*., 2007; Walker *et al*., 2005; Singh *et al*., 2012) as well as by many meta-analysis studies (Kogevinas *et al*.,1997; Burns *et al*., 2001; Collins *et. al*., 2009; Ruder *et al*.,2005; Manuwald *et al*., 2012; Collins *et al*., 2009). People who are handling these hazardous chemicals get exposed through the skin or by the respiratory routes. The effects have been confirmed in different agricultural (Bonner *et al*., 2017; Alavanja *et al*.,2004) and epidemiology (Boffetta *et al*., 2011) studies.

Air pollution

Lung cancer is no more called a smoker's disease as air pollution is emerging as a major cause in both men and women. In urban areas incidence of lung cancer is more as compared to those in rural areas (Cohen *et al*., 2000). Air pollutants are released in the air from mining, industries, vehicles, municipal waste and incomplete incineration from houses (EPA, 2001). The emission scenario indicates that by 2050, the contribution of outdoor air pollution to premature mortality on a global scale will become double (Lelieveld *et al*., 2015). Smoke inside the home is also an important determinant (Sapkota *et al*., 2008). Incomplete combustion of coal from households releases cancer triggering factors like, carbon monoxide, benzene, formaldehyde, and polycyclic aromatic hydrocarbons in the environment (IARC; Raspanti *et al*., 2016).

Asbestos

Asbestos is naturally occurring fibrous silicates which are carcinogenic and result in serious risk of developing lung cancer and mesothelioma (Uguen *et al*., 2017). Environmental Protection Agency (EPA) has classified asbestos as Group A carcinogen to humans (US Environmental Protection Agency, 2014). Mc Donald *et al*. in 1980 (Mc Donald *et al*. 1980) reported the effect of asbestos on workers who had worked in the Quebec chrysotile mines and mills in Canada for at least one month. Chrysotile is a type of asbestos. 244 workers died due to lung cancer between 1891 and 1920 in this place. After that, there have been many epidemiological studies available on asbestos-exposed workers (Frost et al. 2011). Despite these studies, its use has continued in many Asian countries with India being a major consumer of asbestos after China (Kazan-Allen L.,2007). All asbestos types were associated with lung cancer (Nielsen *et al*., 2014). A study on asbestos exposure to Italian shipyard workers showed that chrysotile was the most common type of asbestos fibre in asbestos-related lung plaques (Merlo *et al*., 2018). The size of asbestos fibre also varies and most of the carcinogenic fibres were found to be of 8 mm in length (Kagan, 2013).

Passive smoke

The role of tobacco smoking has been repeatedly linked to lung cancer in humans (Butler *et al*., 2017; Sun *et al*., 2017). The person who does not smoke can be affected by passive smoking and develop many respiratory disorders and lung cancer (US EPA, 1993). Those who had never smoked, both among men and women have shown histology of adenocarcinoma, a type of lung cancer (Das *et al*., 2017). Many studies in non- smoker women and in children linked these cases to second- hand smoking (Rachtan J.,2002; Rapiti *et al*.,1999).

Metals: There are shreds of evidence that exposure to certain metals increases the risk of lung cancer development (Rapiti *et al*.,1996). Environmental and occupational exposure to arsenic increases the risk of this disease. Smelters and those who work in ore mines and inhale dust from lead, gold, and copper and are the major targets of occupational exposure (Lubin *et al*., 2008). Hard metals like pseudo alloys of cobalt and tungsten carbide and other metallurgical products many of which contain cobalt also linked to lung cancer (Wild, 2000). A case-control study of 76 lung cancer deaths and 368 controls in an endemic area for Blackfoot disease in Taiwan was carried out to assess the effect of drinking water arsenic content on lung cancer (Chen *et al*., 1962). High concentration (>100ug/L) of arsenic in drinking water is associated with lung cancer (Cantor *et al*.,2007). Many studies also indicate the role of occupational exposure to nickel (Chiou *et al*., 2014; Grimsrud *et al*., 2002; Bernacki *et al*., 1978) and beryllium (Mosquin *et al*., 2017; Levy *et al*., 2009; Schubauer-Berigan *et al*., 2011; Agency for Toxic Substances and Disease Registry,2014; World Health Organization, 2012).

Ionizing radiation

The role of ionizing radiations in cancers has been established for a long time (Doll R.,1995). Ionizing radiations like radon, gamma and x-rays are emitted from radioactive materials present in natural sources like soil, water, rocks, and air. Workers of underground mines are exposed to radon and many studies provide evidence that these workers developed lung cancer (Field *et al*., 2000; Alavanja *et al*., 1999; Krewski *et al*., 2006). Uranium contamination is often cited as a risk factor in ionizing radiation epidemiology (Tirmarche *et al*.,2004).

Environmental factors cause mutations in lung cancer

Lung cancer mutations are associated with smoking and other environmental factors (Kawaguchi *et al*., 2015). Oncogenic driver mutations in P53 and KRAS increase proportionally with smoking status, whereas mutations in EGFR and SMAD4 decrease (Varghes *et al*., 2013). A study on uranium mine workers to see the effect of radon on a very rare mutation in lung cancer showed that 31% of the 52 squamous cell or large-cell carcinomas presented AGG to ATG transversions in the codon 249 of TP53 (Taylor et al., 1994; McDonald *et al*., 1995). Taken together, exposure to environmental risk factors for lung cancer does affect molecular profiles, however further studies may be required in this area.

Interventions to prevent exposure to environmental determinants

People are exposed to environmental risk factors for cancer in homes, schools, and workplaces. Large scale population-based interventions need to be done for the prevention of lung cancer so that they can reach many people in a given country. A range of regulations at the national and international level are made to reduce exposure to chemical and physical exposures to environmental carcinogens (Table 1). US Environmental Protection Agency (EPA) has regulated emissions from households by giving EPA certified wood heater models (US Environmental Protection Agency,2008). Taking into consideration tobacco smoke as an environmental carcinogen, the Government of India has implemented "Cigarettes and Other Tobacco Products Act" in 2003 which prohibits smoking of tobacco in public places (Govt of India, 2003). Food Agriculture Organization (FAO) has set minimum pesticide levels on food products to reduce chemical exposure (World Health Organization, 2008). National Institute of Environmental Health and Safety has made many policies on reducing the exposure of hazardous metals like arsenic, beryllium, chromium, nickel (National Institute of Environmental Health and Safety, 2016).

Table1. Interventions for the environmental determinants of lung cancer.

Risk factor	Country	Organization	Intervensions
Chemical exposure	–	Food Agriculture organization (FAO)	Minimum pesticide level on food products
		TURA	Control on toxic chemicals elimination by industries (Ansaldi *et al*.,2011)
	India	-	Food Safety and Standards Act: showing % of ingredients on food product packets (Bhushan *et al*.,, 2013)
Outdoor air pollution	India	Union cabinet, government of India	Special purpose: vehicle, FasalAvsheshUpyog Nigam (FAUN), to serve as a standalone institutional mechanism to deal with crop residue
			Municipal Corporation of Delhi (MCD) and Government of NCT of Delhi: implement a policy for tagging vehicles, set up a green police force for metropolitan cities
		Ministry of Agriculture of India	NPMCR: Promotes technologies for in situ management of crop (Agricoop. Nic.in 2020), financial support to related research
Asbestos	USA	National Institute of Environmental Health and Safety	Consumer Product Safety Commission (CPSC): banned respirable asbestos (www.cpsc.gov)
			Department of Transportation (DOT): gave guideline for transportation [www.transportation.gov]
			Safe Drinking Water Act: MCL 7 million/L80 (Safe Drinking Water Act, 2020)
			Toxic Substances Control Act: identify asbestos in public area for public places (National Toxicology Program , 2019)
Passive smoking	USA	NIOSH	Exposure reduced to feasible level (National Institute of Environmental Health and Safety, 2016)
	India	–	COTPA; 2003: banned smoking at public places [Government of India,2003]
Arsenic	USA	National Institute of Environmental Health and Safety	National Emission Standard Act: TQ 15,000 lb (U.S. Department of Health and Human Services, Public Health Service, 2016)

			National Institute for Occupational Safety and Health (NIOSH): immediately dangerous to life or health (IDLH) limit =5 life mg/m3 for inorganic compounds (as As). Ceiling recommended exposure limit =0.002 mg/m3 (15 min) for inorganic compounds (as As)
Beryllium	USA	National Institute of Environmental Health and Safety	Food and Drug Administration (FDA): max limit 0.004 mg/L
			OSHA: acceptable peak exposure =0.025 mg/m3 (30-min maximum duration per 8-h shift); ceiling concentration =0.005 mg/m3; permissible exposure limit (PEL) =0.002 mg/m3
			NOISH: IDLH limit =4 mg/m3; ceiling recommended exposure limit =0.0005 mg/m3
Chromium	USA	National Institute of Environmental Health and Safety	Food and Drug Administration (FDA): 0.1 mg/L in drinking water
			OSHA: workers' exposure on an average of 0.005 mg/m3 chromium (VI), 0.5 mg/m3 chromium (III), and 1.0 mg/m3 chromium (0) for an 8 working hour workday
Nickel	USA	EPA	National Emission Hazard Act: TQ 1,000 lb
			OSHA: PEL =1 mg/m3 for elemental nickel and compounds other than nickel carbonyl; =0.001 ppm (0.007 mg/m3) for nickel carbonyl
Ionizing radiation88	USA	–	Public Health Service Act (PHSA): measure environmental radiation level
			Atomic Energy Act (AEA): Safe storage/disposal of radioactive waste

However, there are many countries where no prevention policy is made by the government for occupational lung cancer (Jilcha *et al*.,2016). Exposure to environmental risk factors at the workplace can be regulated by the use of protective equipment for workers and decontamination facilities in industries. Proper training for handling of chemicals in agriculture and trade should be given to the workers. Interventions at the individual as well as government levels are needed to reduce the risk of developing lung cancer due to environmental pollution.

Conclusion

Environmental and occupational interventions are necessary to prevent lung cancer. Tobacco smoking is the primary factor for lung cancer development; therefore, behavioural counselling is needed to avoid smoking. As environmental factors can cause alteration in genes on prolonged exposure, so we need to identify the population at risk to develop lung cancer. Lung cancer in non-smokers is of great concern so other environmental factors need to be taken care of. Policies for banning some metals need to be made as asbestos is still being used in many developing countries. Concluding, in a present scenario not only smoking but other occupational and environmental factors should be considered as a major risk and adequate steps should be taken.

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FERMENTATION OF PRIMARY SLUDGE AT MULTIMILLION LITRES SCALE AND VOLATILE FATTY ACID PRODUCTION FOR BIOLOGICAL NUTRIENT REMOVAL

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Abstract
Biological nutrient removal is a multimillion liters scale biotechnological process operated across the world. Volatile fatty acids (VFAs) produced by fermentation of primary sludge are important part of the modern day biological nutrient removal processes. Anoxic heterotrophic denitrification can be promoted by using these VFAs as external electron donors. Similarly, polyhydroxyalkanoates (PHAs) synthesis can be enhanced by introducing VFAs as external carbon source. Later in the aerobic phase of biological phosphorus removal process, these PHAs are utilized by phosphate accumulating organisms (PAO) for intracellular phosphorus accumulation and removal. This article critically reviews two years' (2014 & 2015) weekly collected data of a full scale (1.745 million liters) fermenter being used for VFAs production by fermentation of primary sludge. Average VFAs production was around 440.7 ± 208.5 mg /L, out of which 44.21 ± 4.02% were acetic acid and 41.15 ± 5.66% were propionic acid. It has been concluded that there is a liner correlation (R2=0.93) between acetic and propionic acid concentration in the fermenter supernatant and a static fermenter is more suitable for propionic acid rich VFAs production. Oxidation of propionic acid has been proposed as the dominant mechanism of acetic acid production. Co-digestion of primary sludge with crude glycerol, hydrolysis of nonliving fraction of the primary sludge, combined hydrogen and VFAs production and pH, DO and hydrogen partial pressure based metabolic shifts are identified as potential strategies for improved VFAs production.

Keywords
Acetic acid; crude glycerol; denitrification; hydrogen production; phosphorus removal; primary sludge; propionic acid; volatile fatty acid.

Introduction

Anthropogenic release of nitrogen and phosphorus into various natural water bodies results in eutrophication (Sun et al., 2010). As a consequence of excess release of nutrient rich materials into the environment, water quality deteriorates and it poses a risk for the receiving ecosystem as well as animal and human health. Phosphorus driven toxic cyanobacterial bloom is one such example (Blackall *et al*., 2002). Being limiting nutrients of aquatic ecosystems, nitrogen and phosphorus removal from the wastewaters has been considered as an effective method to deal with environmental pollution (Blackall *et al*. 2002; Conley *et al*., 2009; Zhang *et al*., 2013).

There are various chemical and biological methods capable of removing nutrients from the wastewaters.

Biological nitrogen removal by autotrophic nitrification and subsequent heterotrophic denitrification releases harmless nitrogen gas which makes it a mostly used and economically efficient method (Sun *et al*., 2010). Although phosphorus can be removed by chemical precipitation technique, a trend towards increase dependency on biological phosphorus removal can be observed across the wastewater treatment plants. Low cost operation and reduction in sludge volume are considered to be the two most important advantages of biological phosphorus removal process over chemical precipitation (Helness, 2007). Traditional activated sludge processes with limited phosphorus removal capacity have been going through modernization or replacement to accommodate an efficient enhanced biological phosphorus removal (EBPR) process (Blackall *et al*., 2002).

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Biological nitrogen removal and EBPR can be simultaneously carried out in the same facility with appropriate reactor design. Availability of readily degradable carbon source is a common prerequisite for successful biological removal of both nitrogen and phosphorus. Anoxic heterotrophic denitrification pocess involves reduction of nitrate and nitrite and requires an electron donor in the form of biologically available carbon source (Sun *et al*., 2010). Likewise, as a part of EBPR process, under anaerobic condition heterotrophic phosphorus accumulating organisms uptake bioavailable carbon rich organic compounds and convert them to polyhydroxyalkanoates (PHAs). Then in the subsequent aerobic stage of phosphorus removal process, these stored PHAs are used for normal cellular activities as well as for phosphorus accumulation (Zhang *et al*., 2013). Since the amount of usable carbon source present in the wastewater is not sufficient to meet nitrogen and phosphorus removal targets, supply of an external carbon source is exclusively considered. Initially methanol and acetate used to be the carbon sources of choice for biological nutrient removal (Ra *et al*., 2000). Later volatile fatty acids (VFAs) produced by multimillion liters scale fermentation of primary sludge have replaced these compounds as an economically favorable alternative (Rabinowitz, 2014). Primary sludge is the material of choice for VFAs production because it is readily available in large quantity within the wastewater treatment facility and rich in easily fermentable materials.

Critical analysis of the data obtained from these multimillion liter scale fermenters is a crucial step for new discoveries and constant evolution of the process. At the same time, recent findings of fundamental small scale studies on this topic should be compared with the actual process being used in the wastewater treatment plants. This article presents an analysis of two years' (2014 and 2015) data of a 1.745 million liters scale fermenter being used for VFAs production. These data include variation in influent primary sludge composition, characteristics of the fermented supernatant and corresponding change in the properties of the fermented primary sludge. Based on this analysis and considering the recent advances in this field, certain recommendations have been put forward for future investigations.

1. Importance of VFAs in nutrient removal from wastewater

Heterotrophic denitrification

Biological nitrogen removal is a common process of nitrogen removal from wastewater (Carrera *et al*., 2003). Conventional biological nitrogen removal process involves sequential nitrification and denitrification steps (Peng and Zhu, 2006). Nitrification is the process where ammonium and other reduced form of nitrogen are oxidized to nitrite and nitrate (Ward *et al*., 2011). Although, heterotrophic nitrification is also known; autotrophic ammonium oxidizing bacteria are mostly responsible for this process (Oguz, 2004; Zhang *et al*., 2011). Based on the microorganisms involved, nitrification process could be either aerobic or anaerobic (Kim *et al*., 2005). Likewise, denitrification is a reduction process which can be aerobic, anoxic and anaerobic (Elefsiniotis and Li, 2006; Kim *et al*., 2005; Oguz, 2004). However, it is mostly observed in the absence of oxygen and basically an anoxic process (Calderer *et al*., 2010; Yatong, 1996). Heterotrophic denitrification is commonly used in most wastewater treatment facilities (Lim *et al*., 2006). Denitrifying organisms are mostly facultative anaerobic bacteria capable of shifting their metabolic activities according to the environmental conditions (Calderer *et al*., 2010). Some species of Achromobacter, Alcaligenes, Denitrobacillus, Micrococcus, Paracolobactrum, Pseudomonas and Spirillum are known to have heterotrophic denitrification ability (Lebesgue and Miglioretti, 1981). This process requires a carbon and electron source in the form of easily biodegradable organic substance (Calderer et al., 2010; Lim et al., 2006). It is used in new biomass synthesis and to fulfill energetic requirements (Yatong, 1996). In fact, presence of readily biodegradable carbon source is the major limiting factor of heterotrophic denitrification process which, in turn, can control the effluent quality (Ucisik and Henze, 2008). Typical municipal wastewater has low COD and thus it is difficult to achieve required denitrification activity by directly subjecting it for denitrification.

In order to avoid this situation, an external carbon source can be directly added to the denitrification reactor or it can be generated in the same wastewater plant (Lim *et al*., 2006). Methanol is known to be used as an external carbon and electron source for denitrification process; however, it is expensive (Elefsiniotis and Li, 2006; Lim *et al*., 2006). Alternatively, onsite volatile fatty acids (VFAs) production by fermentation of primary sludge and VFAs mediated denitrification has become popular for municipal wastewater treatment. A schematic representation of this denitrification strategy has been presented in Figure 1. Here VFAs acts as electron donor and nitrate acts as terminal electron acceptor (Calderer *et al*., 2010). A C:N ratio of 2:1 was found to be capable of complete denitrification when acetic acid, propionic acid or a 1:1 mixture of both VFAs were used as carbon sources (Elefsiniotis and Li, 2006). It has been observed that during denitrification acetic acid is utilized faster than propionic acid (Elefsiniotis and Li, 2006). Butyric acid and propionic acids are consumed only after a portion of acetic acid is utilized, which is followed by valeric acid utilization (Elefsiniotis *et al*., 2004). Other organic carbon sources with a relatively complex structure are the last choices as carbon sources and electron donors (Elefsiniotis *et al*., 2004). Therefore, sufficient amount and appropriate composition of the VFAs mixture is crucial for heterotrophic denitrification.

Phosphorus removal

As an environmentally friendly alternative of alum or lime based phosphorus removal by chemical precipitation, enhanced biological phosphorus removal (EBPR) has become popular from around 1975 (Comeau *et al*., 1986; Mulkerrins *et al*., 2004). Now-a-days the process has been

operated at multimillion liters scale for wastewater treatment around the world. However, still it has been going through modernization in terms of improved efficiency and cost effectiveness. As shown in figure 2, the reactor system used for the process has an anaerobic zone and an aerobic zone. In the anaerobic zone, readily available carbon sources are consumed by the microorganisms with subsequent release of phosphorus to the medium (Sathasivan, 2009). During this stage consumed carbon sources are stored as polyhydroxyalkanoates (PHA) such as polyhydroxybutyrate (PHB) (Sathasivan, 2009; Smolders *et al*., 1995). Once the sludge moves to the aerobic zone with limited carbon source, phosphorus removal takes place by utilizing the stored PHB (Figure 2) (Comeau *et al*., 1986). This phase is characterized by the decrease of intercellular PHB level with simultaneous decrease in phosphorus concentration in the medium (Seviour *et al*., 2003). Removed phosphorus is subsequently stored as intracellular polyphosphate (Mulkerrins *et al*., 2004). The amount of phosphorus removed from the wastewater and stored as polyphosphate could be as high as 15% of dry biomass (Seviour *et al*., 2003). Thus, at the anaerobic phase of the process, the medium/wastewater should have sufficient amount of readily biodegradable carbon source for PHB synthesis.

VFAs have been found to be suitable carbon source for this purpose (Mulkerrins *et al*., 2004; Randall *et al*., 1997).

In fact, a correlation could be found between the amount of VFAs in the medium and phosphorus accumulation capability of the microbial population of the sludge (Randall *et al*., 1997). It has been determined that for each mg of phosphorus removal, around 20 mg acetic acid equivalent COD is required (Abu-ghararah and Randall, 1991). Branched isomers of butyric acid and valeric acid were found to be more effective in phosphorus removal compared to their normal counterparts (Abu-ghararah and Randall, 1991). Between acetic acid and formic acid, the former is considered to be an effective VFA for EBPR process, whereas formic acid may not have any role (Jeyanayagam, 2005). Since municipal wastewater has relatively low COD, addition of VFAs in the anaerobic phase is required for effective phosphorus removal. In this context, fermentation of primary sludge for large scale VFAs production and release of these VFAs in the anaerobic zone of an EBPR process has been widely considered (Chu and Mavinic, 1998; Pitman *et al*., 1992). Depending on the amount of VFAs externally added to the system and biodegradability of influent COD, sludge retention time (SRT) of the anaerobic zones can be varied to optimize PHB accumulation and phosphorus removal (Sedlak, 1991). Similarly, based on the nitrate level of the medium, SRT of the aerobic zone can be varied to achieve proper nitrification and phosphorus accumulation (Grady *et al*., 2011). Thus, large scale VFAs production has become an integral part of wastewater treatment process across the municipalities.

2. Fermentation of primary sludge for VFAs production

As it is evident from the above discussion, VFAs are preferred carbon source for enhanced biological phosphorus and nitrogen removal process. These molecules can be easily transported through the cell membrane and hence they can be quickly used by the heterotrophic microorganisms (Banister and Pretorius, 1998). Primary sludge generated during the wastewater treatment process needs not to be collected and transported to the wastewater treatment plant for VFAs production. This is its biggest advantage over any other feedstock capable of producing VFAs. Likewise, this approach reduces the sludge volume. Between primary sludge and activated sludge generated during municipal wastewater treatment, the former has better VFAs production ability (Ucisik and Henze, 2008). On the contrary, the amount of activated sludge generated during the process is more, thus a larger amount of VFAs could be produced by fermentation of activated sludge (Ucisik and Henze, 2008). However, fermentation of activated sludge may release already accumulated phosphorus back to the medium; thereby overall benefit might be reduced.

Moreover, biodegradation of activated sludge is relatively more complicated and it may require hydrolysis or pretreatment (Ucisik and Henze, 2008). Therefore, VFAs production by fermentation of primary sludge has been practiced at multimillion liters scale for treatment of real municipal wastewater.

In figure 3 (a) total volatile suspended solid percentage of total suspended solid found in the supernatant of the primary sludge fermenter has been presented. The data shows that total volatile suspended solid found in the supernatant is around 90.07 ± 4.36 % of total suspended solid. It indicates that the suspended solid of the supernatant is mostly composed of digestible material and considered to be suitable for fermentation. In figure 3 (b) the volatile solid percentage of total solid of the fermenter sludge has been presented. Always around 85.7 ±3.17 % of total solid was found to be volatile solid. Likewise, in figure 4 (a & b) respectively the total COD of fermenter supernatant and fermenter sludge has been presented. From the figures it can be concluded that total COD of the supernatant can be between 1-4 g/L whereas, the same of fermenter sludge could be around 100-150 g/L and in some instances more than 200 g/L. From these data it is evident that primary sludge is a huge digestible carbon source and a right choice for VFAs production.

Characteristics of primary sludge

Primary sludge is the product of primary treatment of wastewater treatment process involving primary clarifiers. It is composed of the suspended solid and other heavy materials accumulated in the bottom of the primary clarifier which are separated from the liquid fraction of the wastewater by gravitational sedimentation. Composition of the primary sludge may vary from one treatment plant to another or according to the seasons of the year. In Table 1 different reports of primary sludge characterization have been

summarized. From the table it can be concluded that total COD of primary sludge could be around 11 to 45 g/L. Likewise, total solid (TS) could be around 26 to 38 g/L and total volatile solid (VS) could be around 9 to 25 g/L, whereas pH may vary between 5.4 to 6.7.

\In figure 5, 6 and 7, respectively TKN, total phosphorus and total solid profiles of primary sludge of Pine creek wastewater treatment plant (Canada) for the period of January 2014 to December 2015 have been provided. In general, over these two years each week sludge samples were collected from the bottom of the primary clarifier and analyzed for TKN and total phosphorus. The results show that the amount of TKN in the primary sludge can vary between 75 to 1000 mg/L, likewise; total phosphorus content may fluctuate between 10 to 250 mg/L and total solid may vary from 0.5 % to 3.5% (w/w). Thus, for effective fermentation of primary sludge, the process must be robust enough to withstand this variation of influent sludge composition.

Simultaneous fermentation and sludge settling

Simultaneous fermentation and primary sludge settling is a VFAs production strategy mostly applied in modern wastewater treatment plants. In addition of producing VFAs to complete the biological nutrient removal process, this approach can simultaneously minimize the sludge volume by further concentrating or thickening the sludge. Moreover, it can save energy and reduce the number of required reactor tanks by bypassing a complete mix tank for fermentation. It has been observed that if the sludge is not mixed during its fermentation, depending on sludge type VFAs yield could be increased from 0.04 to 0.07 mg VFAs (COD)/mg COD or from 0.09 to 0.15 mg VFAs (COD)/mg COD (Banister and Pretorius, 1998). Fermentation using a complete mix tank might expose the microorganisms to the process inhibitors such as the products (VFAs) or might facilitate further metabolization of the products (Banister and Pretorius, 1998). In Table 2 different reports on primary sludge fermentation have been summarized. Most of these studies were conducted under complete mix conditions and cumulative VFAs productions reported were around 530 mg acetic acid equivalent /L to 3570 mg acetic acid equivalent /L. This indicates that although VFAs yield could be decreased due to mixing, still considerable amount of VFAs could be produced by this technology. Therefore, VFAs production by fermentation of primary sludge is not completely dependent on whether mixing is provided or not. Other factors such as total solid and total volatile suspended solid concentration of the feed, HRT, SRT are also responsible in determining the cumulative production of VFAs. Hence, better VFAs yield should not be the only reason behind choosing a static fermenter over complete mix tank.

From figure 5 it has been observed that over a period of two years TKN value of the primary sludge was within around 75 to 1000 mg/L, whereas, TKN of fermented sludge of the static fermenter was found to be around 1200 to 3500 mg/L. As shown in figure 6, total phosphorus content of primary sludge was between 10 to 250 mg/L, whereas, the same of the fermented sludge of static fermenter was found to be around 300 to 600 mg/L. Similarly, as shown in figure 7, total solid of the primary sludge was around 0.5%-3.5% (w/w) and after fermentation and simultaneous sludge thickening it became around 6 to10 % (w/w) in the fermented sludge. Thus, it could be concluded that as a result of fermentation and simultaneous sludge thickening; nitrogen, phosphorus and solid were concentrated within the sludge. Therefore, smaller footprint due to reduction of sludge volume and possible energy saving are the two most probable reasons behind selecting static fermenter over complete mix tank in relatively new wastewater treatment plants of the same city, as in the present case.

VFA profiles

Detailed VFAs profile obtained by primary sludge fermentation using a 1.745 million liters fermenter over the period of two years has been provided in figure 8. In general, samples were taken once a week with certain exceptions and analyzed by GC. Average VFAs production recorded for this period was around 440.7 ± 208.5 mg /L, where acetic acid (44.21 ± 4.02%) and propionic acid (41.15 ± 5.66%) were the dominant products followed by butyric acid (9.34 ± 2.50%) and valeric acid (3.51 ± 0.60%). This amount is equivalent to 383 ± 178.11 mg acetic acid /L. It has been reported that acetate concentration of around 400 mg/L in the influent stream is suitable for anaerobic phosphorus release and its subsequent aerobic uptake (Mulkerrins et al., 2004). On the contrary, more than 600 mg/L of acetate in the influent stream was found to be inhibitory for both phosphorus release and uptake (Mulkerrins et al., 2004). It suggests that the amount of VFAs produced in the present large scale fermenters of the wastewater treatment plant by fermentation of the primary sludge is sufficient for effective phosphorus removal. However, as per the configuration of the present wastewater treatment plant, VFAs produced in two fermenters, each of 1.745 million liters capacity, are fed to two bioreactors, each of 25 million liters capacity for phosphorus and nitrogen removal. Therefore, VFAs are considerably diluted in the bioreactors. It suggests that there is a scope for increasing the VFAs concentration of the fermenter supernatant well beyond presently achieved 440.7 ± 208.5 mg /L.

Study suggests that among different carbon sources including VFAs, acetate is most effective electron donor for denitrification (Elefsiniotis *et al.*, 2004). It has been presumed that compared to other carbon sources such as methanol and butyric acid, acetic acid does not need any enzymatic modification before entering the metabolic pathway and hence it could be directly metabolized (Elefsiniotis *et al.*, 2004). The VFAs production profile obtained from the full scale fermenter being operated in the wastewater treatment plant (Figure 8), shows that propionic acid is nearly 41.15 ± 5.66% of total VFAs produced by fermentation of primary sludge, whereas acetic acid is nearly 44.21 ± 4.02%. Figure 9 (a) shows the correlation between

two years' data of total acetic acid equivalents and propionic acid concentration in the fermenter supernatant. There is a linear correlation (R2=0.99) between these two which indicates the dominance of propionic acid in determining the acetic acid equivalence of produced VFAs. Similarly, as shown in figure 9 (b) there is a linear correlation (R2=0.93) between acetic acid and propionic acid concentrations in the supernatant. This correlation indicates that oxidation of propionic acid to acetate by hydrogen producers could be a dominant part of primary sludge fermentation (Liu et al., 2004). This oxidation process might be perfectly balanced by feedback regulation controlled by real time concentrations of these two acids. In general, acetic acid is the dominant product of primary sludge fermentation in presence of agitation and slow aeration. In the present case since it is a static fermenter, acetic acid production is probably more propionic acid oxidation dependent.

Figure 8 (c) shows that the amount of VFAs present in the fermenter supernatant at any time is only around 25% of its total COD. It means a large amount of COD is still left unutilized in the supernatant and improvement in VFAs production might be possible by enhancing the utilization of such COD. Figure 10 (a & b) shows the correlation between total COD of the fermenter supernatant and total acetic acid and total VFAs present in the supernatant, respectively. The figures suggest that although only about 25% of the total COD is converted to VFAs; total acetic acid and total VFAs content of the supernatant could be increased by increasing the total COD of the supernatant. These correlations observed in the data collected over the two years of study period suggest that the fermenter supernatant must have a fraction of total COD as non-VFAs COD. This non-VFAs fraction of total COD might be essential for the process to maintain its redox balance. For instance, in order to maintain an appropriate NADH/NAD+ ratio; NAD+ is regenerated by reduction of intermediate metabolite such as VFAs to produce reduced end products such as ethanol, butanol, lactate among others (Chen et al., 2009; Liu et al., 2016; Ramey and Yang, 2004; Saini et al., 2016; Tashiro et al., 2004).

On the contrary, maximum utilization of the COD could be ensured by extending the SRT. However, a long SRT of around 8 days may initiate methanogenesis; thereby considerable amount of VFAs will be lost. Keeping this possibility in mind, a SRT of around 3-5 days is considered to be optimum for this kind of process (Chu et al., 1997; Ucisik and Henze, 2008). Thus, COD and SRT are two crucial factors to determine the amount of VFAs produced by fermentation of primary sludge using a static reactor. There is a possibility to increase the VFAs production by increasing the COD of the fermenter supernatant. In this context, co-fermentation of primary sludge with crude glycerol (section 4.2) is an interesting option.

3. Possible improvement of the present process

Combined VFAs and hydrogen production Hydrogen combustion does not generate CO2, water is the major byproduct of this process and hence it is considered to be a green alternative of conventional energy carriers (Rittmann *et al*., 2015; Sarma *et al*., 2015c). Additionally, energy yield of hydrogen is nearly 2.75 times higher than that of commercially available hydrocarbon based energy carriers (Sarma *et al*., 2015c). Because of relatively higher hydrogen production rate, low process cost and the possibility of using large variety of organic wastes as the feedstock; among different biotechnological methods of hydrogen production, anaerobic fermentation is considered to be the most favorable one (Ren *et al*., 2009). Hydrogen production by anaerobic fermentation and VFAs production by fermentation of primary sludge are almost similar processes. During hydrogen production by anaerobic fermentation, volatile fatty acids such as acetic acid and butyric acid are produced as byproducts (Amorim *et al*., 2014; Chen *et al*., 2006; Hawkes *et al*., 2002). For instance, biomass, H2, acetic acid, butyric acid, reducing equivalents and ATP are the final products of anaerobic fermentation of glucose by Clostridium butyricum(Chen *et al*., 2006). In general, about 30–40% of the initial feedstock is used in hydrogen generation; remaining 60–70% are metabolized to form various byproducts dominated by acetic acid, butyric acid and propionic acid among others (Sarma *et al*., 2015c). Production of these metabolites is often considered as a major bottleneck of biological hydrogen production as a considerable amount of feedstock is lost in the form of these products (Kumar Gupta *et al*., 2013; Sinha and Pandey, 2011; Wang *et al*., 2006). Therefore, these VFAs rich unwanted byproducts of hydrogen production process can be converted to valuable resource (Sarma *et al*., 2015a) by combining the hydrogen and VFAs production technologies and applying these products for biological nutrient removal based wastewater treatment processes presently being used throughout themunicipalities.

At present Pine Creek wastewater treatment plant (Canada) has been producing methane rich biogas as a source of renewable energy in two anaerobic digesters of 8.55 million liters scale, by anaerobic digestion of waste sludge. Hydrogen rich biogas to be generated by the proposed approach could be mixed with the methane rich biogas presently being produced by wastewater treatment plants. Calorific power of hydrogen rich biogas is more than that of methane rich biogas (Sydney *et al*., 2014). It has been reported that engine power output and overall performance of methane fueled spark ignition engine can be increased by using a methane-hydrogen blend (Karim *et al*., 1996). Therefore, combined hydrogen and VFAs production by fermentation of primary sludge and blending the hydrogen rich biogas to be obtained by this approach to methane rich biogas presently being produced in the wastewater treatment plant seems to be a win-win combination.

Maintaining appropriate hydrogen partial pressure will be crucial for developing a combined hydrogen and VFAs production technology using primary sludge. An increase in hydrogen partial pressure in the headspace of the fermenter is not favorable for hydrogen production (Bouzas *et al*., 2007; Rajhi *et al*., 2015; Sarma *et al*., 2015b). It shows a temperature dependent response towards hydrogen partial pressure. For instance, hydrogen partial pressure of 50 kPa, 20 kPa and 2kPa were found to be optimum for hydrogen production at 60ºC, 70ºC and 98ºC, respectively (Pandey *et al*., 2013). Therefore, accumulated hydrogen should be removed from the headspace of the reactor in order to facilitate hydrogen production. However, removal of hydrogen should not be allowed to create strict anaerobic condition in the fermenter. Such situation will support methanogenic activities; thereby accumulated hydrogen and acetic acid will be consumed.

Co-fermentation of primary sludge and agro- industrial wastes

Co-fermentation of primary sludge and agro-industrial waste is an area of this subject where further investigation could be focused. Table 3 summarizes various reports on co-fermentation of primary sludge and agro-industrial wastes. As it is evident from the table, co-fermentation of primary sludge and waste activated sludge has been largely considered for VFAs production. In general, waste activated sludge offers an advantage over other agro-industrial wastes a co-substrate since it is produced in large quantity and readily available within the wastewater treatment facility. However, biodegradation of activated sludge may require pretreatment for effective VFAs production and it is a concern for its large scale application (Ucisik and Henze, 2008). Additionally, carbon, nitrogen and phosphorus as well as other pollutants which are already removed from the wastewater as activated sludge and ready for final disposal will be reintroduced to the process by using this substrate for co-fermentation. Likewise, waste activated sludge is rich in protein with a low carbon to nitrogen ratio, largely considered as unfavorable for VFAs production (Feng et al., 2009). Therefore, a waste material with high carbon to nitrogen ratio will be more appropriate as co substrate for VFAs production.

In most modern municipalities, industries are charged a fee for treating their respective wastewaters in the wastewater treatment plants of the city. Sometimes the wastewater from these industries has high COD value. Most food processing industries come under this category. However, once they are released into the main stream of municipal wastewater, they are diluted and by the time they reach the wastewater treatment plant, become ineffective as carbon source. In this context, if such high COD wastewaters could be collected and separately transported to the wastewater treatment plant for co-fermentation with primary sludge, better result could be expected. A separate industrial wastewater collection pipeline network will be a prerequisite of this approach.

Crude glycerol, a byproduct of biodiesel production could be another attractive co-substrate for VFAs production by fermentation of primary sludge. Production of each 100 kg of biodiesel generates nearly 10 kg of crude glycerol as byproduct (Garlapati *et al*., 2016; Santibáñez *et al*., 2011; Sindhu *et al*., 2011). Corresponding to an increase in global biodiesel production, it has been projected that by 2020 global crude glycerol production will be around 41.9 billion liters (Nanda *et al*., 2014). Market price of crude glycerol is around 0.04 to 0.09 $ per pound and the industry has been looking for a sustainable method for its utilization (Nanda *et al*., 2014). Various options for utilization of crude glycerol have been evaluated by the researchers and some of these

Table 1: A summary of the reports on characteristics of primary sludge.

Source	Total COD	BOD	TS*	TSS*	VS*	TKN*	TP*	pH	Ref.
Sewage treatment farm, Melbourne	11 g/L (total organic carbon)	30.8 g/L	6.56%	5.13%	5.0%	-	-	5.4	(Bhattacharya, 1981)
-	17.106 ± 0.768 g/L	-	-	14.48 ± 0.03 g/L	9.05 ± 0.16 g/L (VSS)	-	-	-	(Skiadas *et al*., 2005)
Wastewater treatment plant, Brisbane	30.2 ± 3.2 g/L	-	26.9 ± 2.9 g/L	-	20.7 ± 2.0 g/L	1.3 ± 0.6 g/L	-	5 to 6.5	(Ge *et al*., 2010)
Wastewater treatment plant, Gyungsan City, Korea (Dry season)	44.83± 2.38 g/L	-	38.46± 0.88 g/L	-	25.64± 0.62 g/L	130±16 mg/L (NH4-N)	17± 16 mg/L (PO43--P)	6.67±0.12	(Ahn and Speece, 2006)
Wastewater treatment plant, Gyungsan City, Korea (Rainy season)	33.95± 2.95 g/L	-	29.16± 2.34 g/L	-	17.28± 1.40 g/L	228±52 mg/L (NH4-N)	31±14 mg/L (PO43--P)	6.55±0.18	(Ahn and Speece, 2006)

*TS (Total Solid), TSS (Total Suspended Solid), VS (Volatile Solid), TKN (Total Kjeldahl Nitrogen), TP (Total Phosphorus).

Table 2: A summary of the reports on primary sludge fermentation and volatile fatty acid (VFA) production.

Fermentation conditions					Acetic acid	Propionic acid	Butyric acid	Valeric acid	Total VFAs	Ref.
Fermenter Scale	Mixing	Air or N_2 gas sparging	Tem-perature	pH						
10 L	-	No	20 ºC	5.8 to 7.0 (not controlled)	-	-	-	-	2148 mg/L	(Ubay-Cokgor *et al*., 2005)
10 L	Yes, 150 rpm	No	20 ºC	5.8 to 7 (not controlled)	1385 mg/L	822 mg/L	419 mg/L	294 mg/L	3, 942 mg/L	(Cokgor *et al*., 2006)
19L	Yes	No	20±1 °C	7.10	~91% of tota VFAs	-	-	-	541 mg acetic acid equivalent/L	(Bouzas et al., 2007)
2.77L	Yes	No	20±0.5 °C	5.81 to 6.04 (not controlled)	-	-	-	-	3314 ± 130 mg acetic acid equivalent/L	(Bouzas *et al*., 2002)
5L	Yes, 36 rpm	No	RT (18 to 28°C)	Final pH (4.8 to 5.2)	-	-	-	-	0.09 mg VFAs (as COD)/ mg COD	(Banister and Pretorius, 1998)
5L	No	No	RT (18 to 28°C)	Final pH (4.8 to 5.2)	-	-	-	-	0.15 mg VFAs (as COD)/ mg COD (66.67% increase)	(Banister and Pretorius, 1998)
0.25 L	Yes	No	30°C	7.16	-	-	-	-	SCOD* 4311 mg/L	(Rubal *et al*., 2012)
0.25 L	No	No	30°C	5.20	-	-	-	-	SCOD 3444 mg/L	(Rubal *et al*., 2012)
6 L	Yes, 180 rpm	No	37°C	6.55 to 7.48	-	-	-	-	~10,000 mg acetic acid equivalent /L	(Bien *et al*., 2015)
4L	Yes	Yes, N2	25°C	~6.89	-	-	-	-	530 ± 162 mg acetic acid equivalent /L	(Maharaj, 1999)
6L	Yes	Yes, O_2 flow rate of 0.025 m3/m3•h	55°C	Not controlled	60.4%	19.3%	12.2%	8.1%	3570 mg acetic acid equivalent /L	(McIntosh and Oleszkiewicz, 1997)
1.745 million liters	No	No	-	Not controlled	44.21 ± 4.02%	41.15 ± 5.66%	9.34 ± 2.50%	3.51 ± 0.60%	383 ± 178.11 mg acetic acid equivalent /L; 440.7 ± 208.5 mg total VFA/L	Present study

*SCOD (Soluble COD)

options include: glycerol recovery (Nanda *et al*., 2014; Wan Isahak *et al*., 2015), biohydrogen production (Sarma *et al*., 2012), bioethanol production (Choi *et al*., 2011; Oh *et al*., 2011), 1,3-propanediol production (Szymanowska-Powalowska, 2014; Wojtusik *et al*., 2015), microbial lipid production (Polburee *et al*., 2015; Tchakouteu *et al*., 2015), organic acid production (West, 2012, 2013), glycerol carbonate production (Nguyen and Demirel, 2011) among others. For all these options the final product should be purified from the reaction mixture or the product yield is so low that it is not economically feasible to purify them. Therefore, research works are still going on and these processes need further improvement.

Alternatively, VFAs produced by co-fermentation of crude glycerol and primary sludge could be directly used for biological nutrient removal without any purification, storage, packaging or transportation. The volume of wastewater to be treated worldwide is so large that by this approach it should be possible to accommodate all the crude glycerol produced by the global biodiesel industry. Unlike most of the agro-industrial wastes, crude glycerol does not need to be hydrolyzed prior to its application. This could be another advantage of this concept as it will directly increase the COD of fermenter supernatant (section 3.3). It is a substrate which has mainly emerged after the establishment of biological nutrient removal process, thus its potential application in this process is mostly unexplored. COD of crude glycerol is

around 1120g/L to 1600 g/L (Khanal, 2011; Larsen *et al*., 2013; Viana *et al*., 2012). Therefore, small amount of crude glycerol will be sufficient to supplement the primary sludge for VFAs production. In most cases pH of crude glycerol is reported to be around 6.9 to 12 (Hu *et al*., 2012; Nanda *et al*., 2014; Wen, 2013). It may be possible to maintain the process pH by crude glycerol addition, which is otherwise expected to decrease due to VFAs accumulation.

As shown in Figure 5 & 6, TKN and total phosphorus of fermented supernatant could be around 80-100 mg/L and 20-25 mg/L, respectively. As already discussed, a C: N ratio of around 2:1 is suitable for nitrogen removal; whereas around 20 mg acetic acid equivalent COD is required to remove 1 mg of phosphorus. Therefore, a considerable amount of VFAs is actually used in removal of the nutrients present in the supernatant itself. Fermenter supernatant with low nitrogen and phosphorus is desirable for better nutrient removal efficiency. In this context, co-fermentation of primary sludge with a feedstock with low nitrogen and phosphorus content such as crude glycerol will be advantageous. Thus, the possibility of co-fermentation of primary sludge and crude glycerol for VFAs production and enhanced biological nutrient removal needs to be validated by further investigation. This could benefit the biodiesel industries not only by providing an environment friendly method for

Table 3: A summary of the reports on co-fermentation of primary sludge and agro-industrial waste for volatile fatty acid (VFA) production.

Agro-industrial waste		Primary sludge	Fermentation scale	HRT or total duration	SRT* or total duration	VFA production	Ref.
Type	Concentration						
Grease trap waste	2 kg COD/m^3 (20% of primary sludge)	10 kg COD/m3/d (300 gallon)	1135.62 L	-	-	6 to 8% improvement	(Latimer *et al*., /d 2016)
Waste activated sludge	50 % (1:1 mixture); total suspended solid: 8693 mg/L	50% (1:1 mixture); total suspended solid: 12593 mg/L	0.9L	4d (batch process)	4d (batch process)	~33 % acetic acid	(Su *et al*., 2013)
Waste activated sludge	50% (1:1 mixture)	50% (1:1 mixture)	5L	4d (batch process)	4d (batch process)	1624 mg/L	(Zurzolo *et al.* 2016)
Kitchen food-waste	25 % by weight	75 % by weight	5L	1d	-	6310 mg/L	(Min *et al*., 2005)
Potato-processing industry wastewater	50% (1:1 mixture)	50% (1:1 mixture)	2L	30h	-	624±98 mg/L	(Banerjee *et al.*, 1999)
Kraft juice processing waste, Feta cheese whey waste, waste activated sludge	25 % (Kraft juice processing waste) + 6% (Feta cheese whey waste)	69 % (60% sludge, 40% waste activated sludge)	25L	-	12d	20 mg/L	(Pathak, 2014)
Molasses	750 mg COD/L	9.69 g/L	10 L	8h (batch process)	8h (batch process)	11.98 mg VFA-COD/g VSS/h*	(Zeng *et al*., 2006)

*SRT (Solids Retention Time), VSS (Volatile Suspended Solid).

utilization of its large amount of byproducts but also it may increase the demand and price of crude glycerol.

Hydrolysis/pretreatment of primary sludge

As it is evident from figure 8, only around 20-30% of total COD of fermenter supernatant is VFAs. It means there is a possibility to improve the present process by converting remaining 70-80% of the COD to VFAs. A sustainable hydrolysis technique will be effective in ensuring bioavailability of this COD. For instance, sonication was found to improve the degradability of organic components of primary sludge (Bien *et al*., 2015). Hydrolysis of primary sludge by pulsed electric field was found to enhance the COD conversion to VFAs (Ki *et al*., 2015). However, hydrolysis may release the nitrogen and phosphorus trapped within the primary sludge (Figure 5 & 6). Two years' data shows the TKN and total P present in the primary sludge are usually around 1000 mg/L and 250 mg/L, respectively. Out of these amounts, how much TKN and P will be released by hydrolysis of primary sludge is not known.

Physical or chemical hydrolysis methods may destroy the living microbial cells thereby the benefit of hydrolysis may not be observed in the form of increase in VFAs production. Alternatively, enzymatic hydrolysis of the primary sludge could be more selective and effective in this regard. In this case, the cost of enzymatic hydrolysis of primary sludge could be a problem. Overall, hydrolysis of primary sludge could be a promising option for improved VFAs production. At this point only limited amount of experimental data is available on this topic and further investigation will be beneficial.

\3.4. Metabolic shift strategy

As it is evident from figure 8, full scale fermentation of primary sludge during simultaneous fermentation and sludge thickening resulted in total VFAs production of 440.7 ± 208.5 mg/L. Out of this amount, 44.21 ± 4.02% is acetic acid and 41.15 ± 5.66% of propionic acid. However, reports suggest that in terms of biological nutrient removal efficiency, a VFAs mixture dominated by propionic acid is more suitable. For instance, compared to acetic acid, if propionic acid is used as the carbon source, lesser amount of phosphorus is released in the initial anaerobic half of biological phosphorus removal process and overall nutrient removal is better (Wu *et al*., 2009). Compared to a propionic acid: acetic acid ratio of 0.16, better biological phosphorus removal has been reported for a ratio of 2.06 (Chen *et al*., 2004). Nitrate accumulation was better when acetic acid was the only carbon source, however a relatively high propionic acid: acetic acid ratio was found to be advantageous for overall biological nutrient removal (Li *et al*., 2008). Likewise, better nitrification, denitrification as well as simultaneous phosphorus removal has been observed when the propionic acid: acetic acid ratio was 1.5 (Li *et al*., 2007). From the present data (Figure 8) it is evident that by fermentation of primary sludge at present conditions, a propionic acid: acetic acid ratio of nearly 1:1 could be obtained. In this context, if the process parameters can be optimized in such a way that the metabolic activity of the microbial consortium could be shifted from towards more propionic acid production, better nutrient removal efficiency could beexpected.

Hydrogen partial pressure in the headspace of the reactor is a crucial factor in anaerobic fermentation which can initiate metabolic shift. It has been reported that by increasing the hydrogen partial pressure in the headspace acetic acid production could be reduced and more amount of long chain VFAs could be produced (Bouzas *et al*., 2007). Similarly, by introducing limited airflow a shift in the composition of VFAs towards more amount of acetic acid production could be observed (Chu *et al*., 1996). For instance, an airflow rate of 0.126 V/V/h resulted in around 950 mg/L of VFAs production of which 81% was acetate and 11% was propionate (Chu *et al*., 1994). Therefore, DO concentration of the fermentation medium could be the other potential switch for regulating propionic acid production. In general, it is know that acidic or neutral pH favors butyric acid production, w hile basic pH is favorable for both acetic acid and propionic acid production (Horiuchi *et al*., 2002). Therefore, pH is another parameter needs to be considered for metabolic activity shift of the microorganisms towards propionic acid production. However, there is a thin line between fermentation and anaerobic digestion. Large change in hydrogen partial pressure, DO concentration or even pH may initiate methanogenesis, which in turn may reduce the effectiveness of the fermentation process.

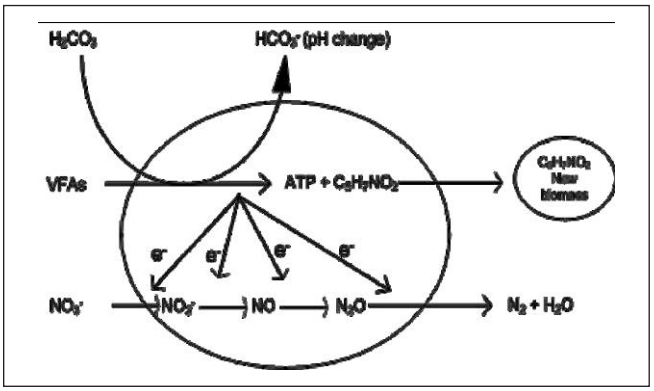


Fig.1: A schematic representation of heterotrophic denitrification in presence of VFAs (Calderer *et al*., 2010; Elsfsiniotis *et al*., 2004)

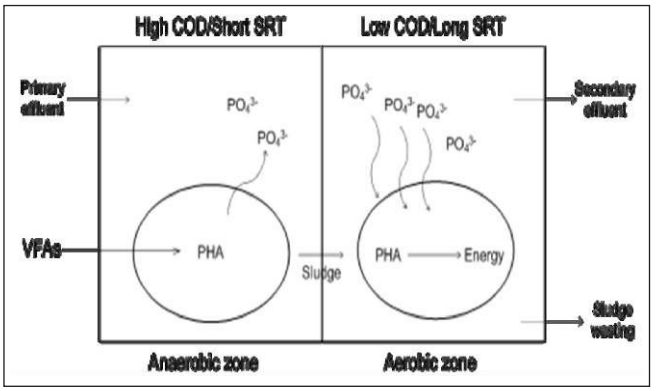


Fig.2: Schematic representation of the VFAs mediated blological phosphorus removal mechanism (Adrianus van Haandel, 2007; Parsons and Smith, 2008; Seviour *et al*., 2003)

Conclusions

Volatile fatty acids production by fermentation of primary sludge has been globally recognized as an economically feasible and efficient method to promote heterotrophic denitrification and enhanced biological phosphorus removal for real wastewater treatment. Simultaneous fermentation and sludge thickening is a latest trend in primary sludge fermentation. Two years' data from a 1.745 million liters scale fermenter being used for simultaneous fermentation and sludge thickening have shown that TKN (1200 to 3500 mg/L) and total phosphorus (300 to 600 mg/L) of fermented primary sludge were always many folds higher than that of influent primary sludge. This is a clear indication that nitrogen and phosphorus were concentrated within the fermented primary sludge.

A complete mixed fermenter could be better than a static fermenter in terms of VFAs productivity. However, it is an energy intensive process and acetic acid is the dominant product in this case. Although acetic acid is considered to be

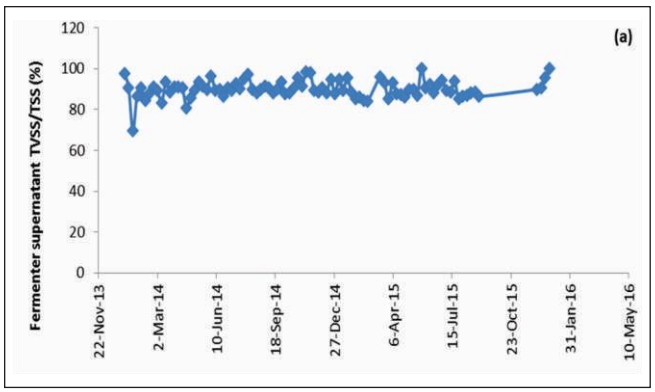


Fig. 3 (a): Volatile suspended solid content of fermenter supernatant (90.07±4.36%) recorded from January 1st 2014 to December 31st 2015).

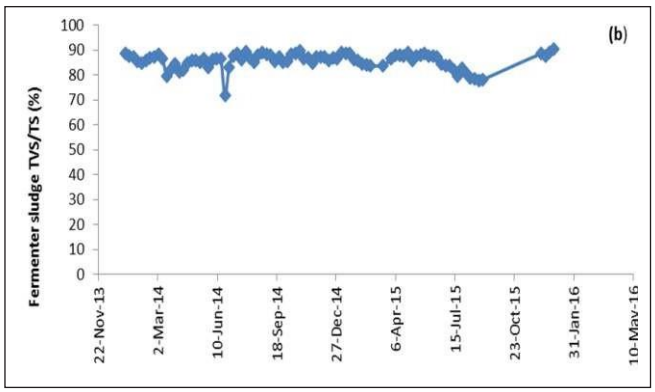


Fig.3 (b) : Volatile solid content of fermenter sludge (85.7±3.17%) recorded from January 1st 2014 to December 31st 2015.

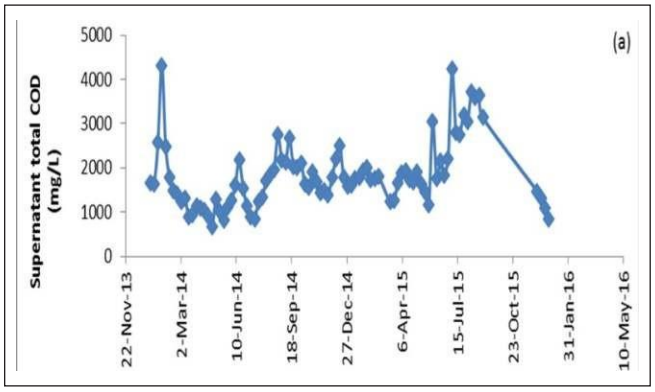


Fig. 4 (a): Total COD profile of fermenter seperastast from 1st January 2014 to 31st Deccember 2015

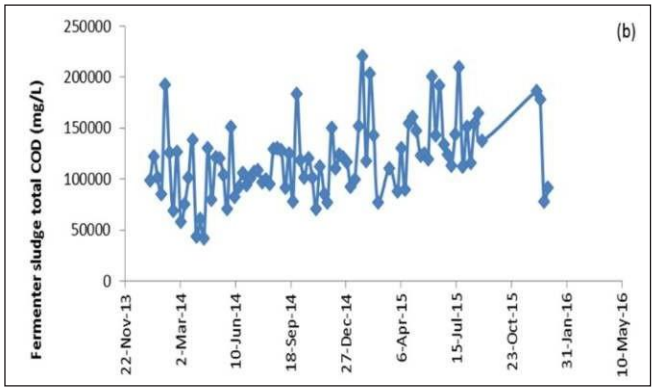
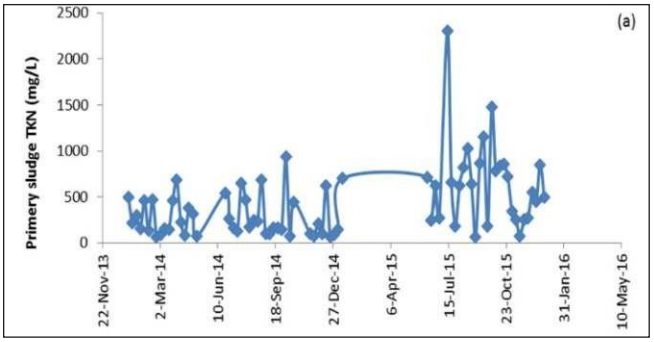
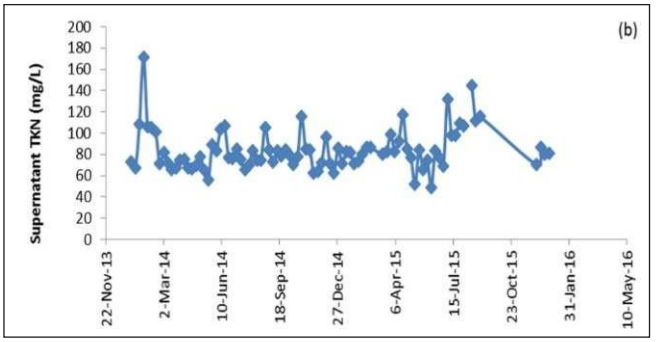


Fig. 4 (b) : Total COD profile of (b) freshwater sludge from 1st January 2014 to 31st December 2015.





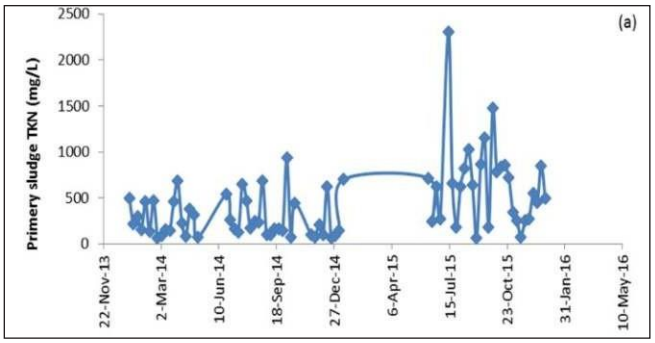


Fig. 5 : TKN profile of primery sludge, freshwater supernatant and fermenter sludge from 1st January 2014 to 31st December 2015.

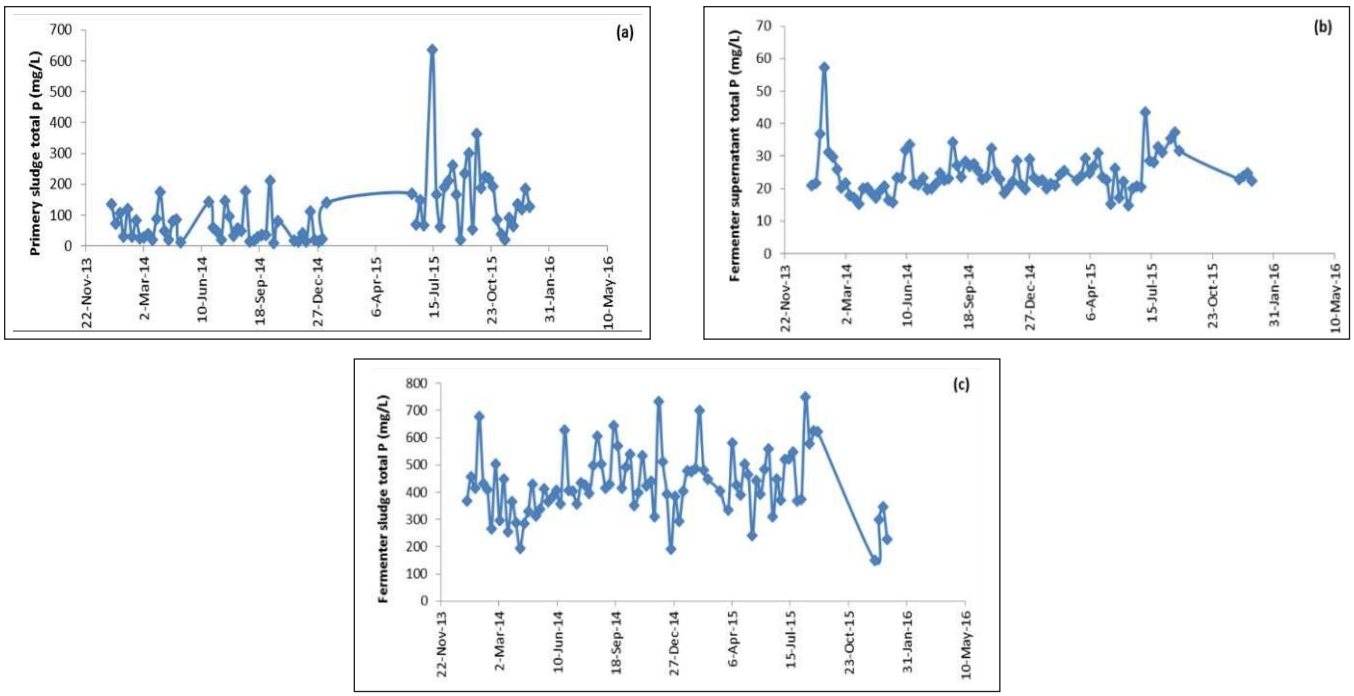


Fig. 6 : Total phosphorus profile of (a) primary sludge (b) fermenter supernatant and (c) fermentersludge from 1st January 2014 to 31st December 2015.

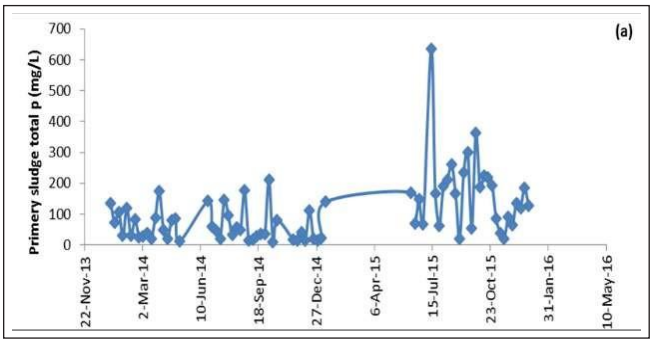


Fig. 7 (a) : Total solid profile of primary sludge from 1st January 2014 to 31st December 2015.

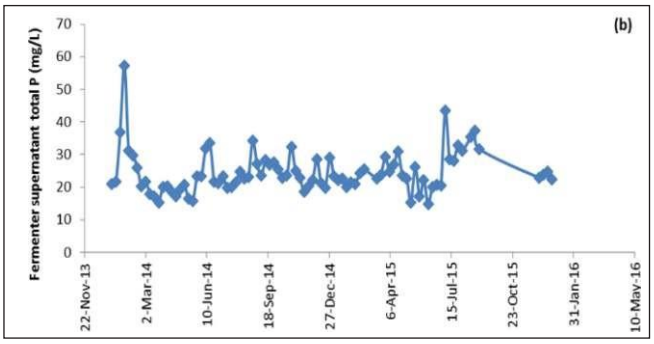
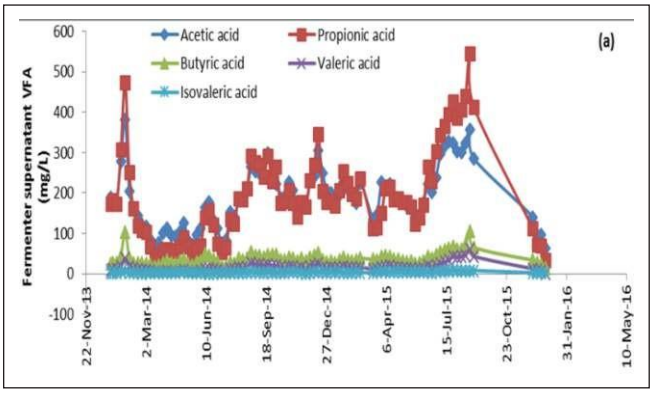
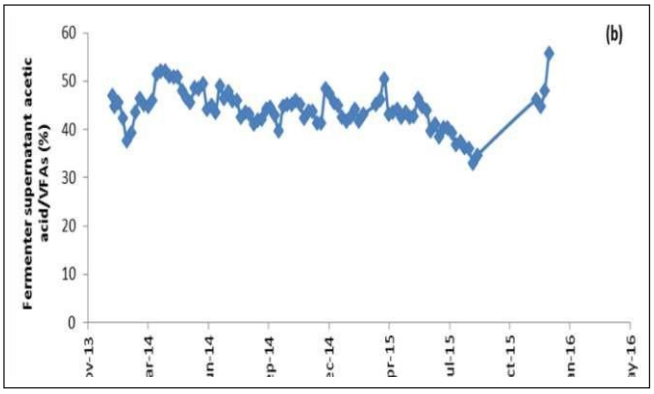


Fig. 7 (b) : Total solid profile of fermenter sludge from 1st January 2014 to 31st December 2015.





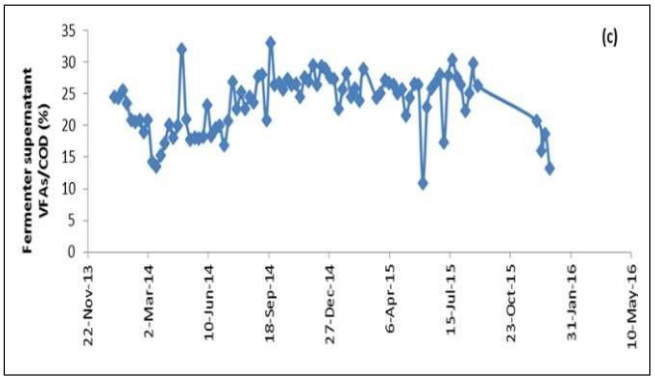


Fig. 8 : (a) Concentration profiles of five different VFAs (b) acetic acid percentage of total VFAs and (c)VFAs percentage of total COD of fermenter supernatant recorded from January 1st 2014 to December 31st 2015.

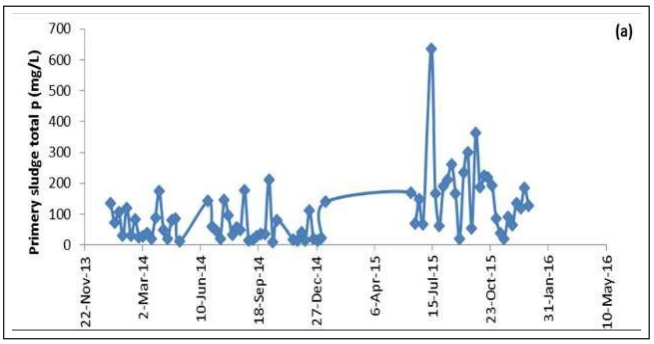


Fig. 9 (a) : Correlation (R^2=0.99) between total acetic acid equivalent and propionic acid concentration in the fermenter supernatant recorded from January 1st 2014 to December 31st 2015

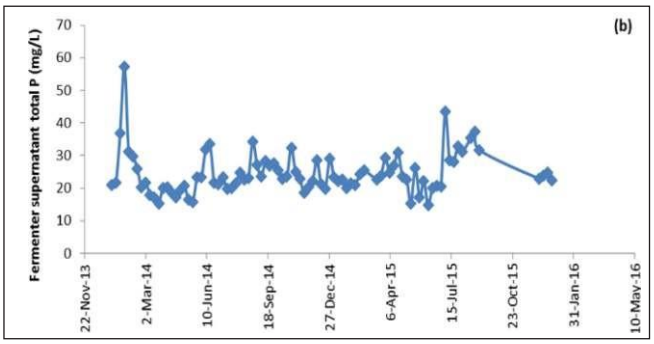
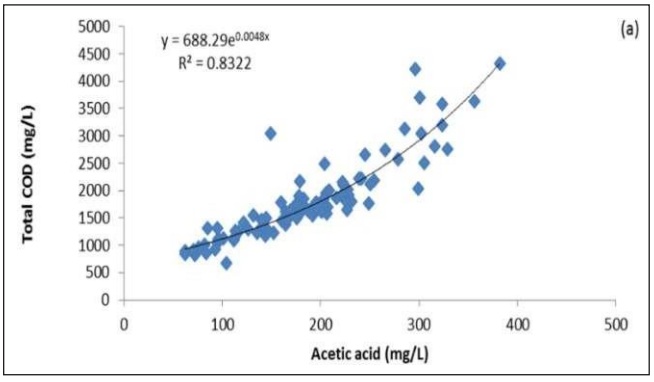
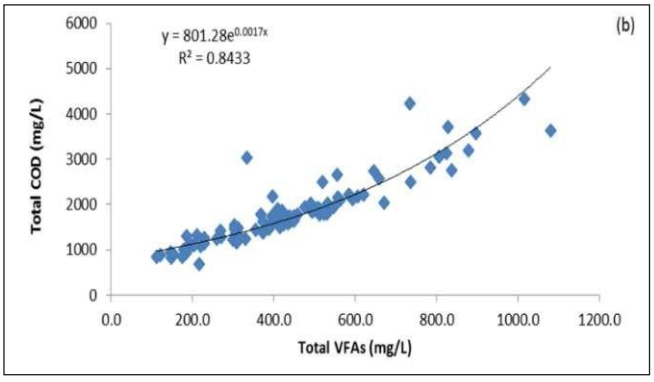


Fig. 9 (b) : Correlation (R^2=0.93) between propionic acid and acetic acid concentrations in the fermenter supernatant recorded from from January 1st 2014 to December 31st 2015





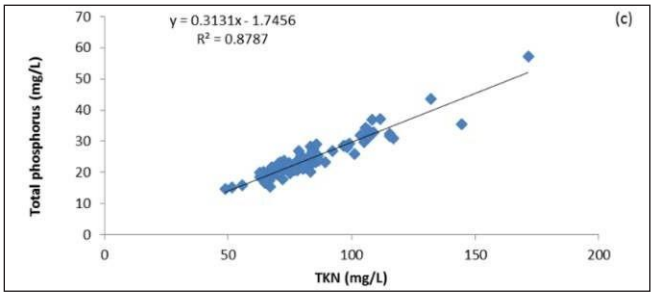


Fig 10 (a) Correlation of (R^2=0.82) between total COD and acetic and concentration (b) correlation(R2=0.84) between total COD and total VFAs concentration (c) correlation (R^2=0.88) between total phosphorus and TKN in the fermenter supernatant recorded from January 1st 2014 to December 31st 2015.

an effective electron donor for heterotrophic denitrificaton, a VFAs mixture dominated by propionic acid is most effective for overall nutrient removal. The large scale fermenter data collected over last two years have suggested that a static fermenter is relatively more favorable for propionic acid production. Unlike complete mixed fermenter, the amounts of acetic acid (44.21 ± 4.02%) and propionic acid (41.15 ± 5.66%) obtained by this process were almost same. There are liner correlations between the concentrations of acetic acid and propionic acid (R2 = 0.93) and total acetic acid equivalent and propionic acid concentration (R2 = 0.99) in the fermenter supernatant. Strongly interlinked metabolic pathways complementing each other could be an explanation of this observation.

Likewise, there is a liner correlation (R2 = 0.88) between total phosphorus and TKN present in the supernatant; however no correlation could be found between the amount of total VFAs or an individual VFA with total phosphorus or TKN of the supernatant. Although phosphate solubilization could be increased by increasing the concentration of VFAs, the amount of total phosphorus and TKN in the supernatant was not a function of VFA type or concentration. Rather the composition of primary sludge seems to be the crucial factor to decide the nutrient content in the supernatant to be used for enhanced biological nutrient removal. Co-fermentation of primary sludge with a feedstock with low phosphorus and nitrogen concentration such as crude glycerol might be an interesting option to cut down the nutrient load of the supernatant.

Simultaneous hydrogen and VFAs production and hydrogen partial pressure based metabolic shift strategy could be considered for producing a VFAs mixture dominated by propionic acid. Aeration and agitation seems to favor a metabolic shift towards increase acetic acid production by fermentation of primary sludge; therefore, a static fermenter looks more appropriate for more propionic acid production. A slightly longer hydraulic retention time might be effective in reducing the DO level and shifting the product profile towards propionic acid.

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GENOMICS AND PROTEOMICS OF *SACCHAROMYCES CEREVISIAE* USED FOR BIOFUEL PRODUCTION

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Abstract
Saccharomyces cerevisiae is the first eukaryotic organism whose genome was sequenced with genomic information of a total of 16 chromosomes. It is being used as a model organism to study the molecular structure and functional characterization of higher eukaryotes. The genomic, proteomic, transcript and metabolic resources are the best platforms to provide information regarding genome, proteins, transcripts and metabolic pathways. The evolution of simple genetics of yeast to genome editing and utilization of genomic resources are summarized in this review. It will give a general overview of various database resources and tools for gathering and generating computational information of yeast. SGD (*Saccharomyces* Genome Database) is an open resource for comprehensive biological integrated information for *Saccharomyces* cerevisiae and helps in finding the target gene such as ADH (alcohol dehydrogenase) for improving biofuel production enhancement. Genome-wide homology annotation also helps in identifying genes and ORFs and also in assigning functions. The potential utilization of these resources may overcome the findings of a target for gene discovery and their use in industrially relevant gene modifications.

Keywords
Saccharomyces cerevisiae, Genetics, Genomics, Proteomics, Transcriptomics, Metabolomics.

Introduction

Saccharomyces cerevisiae represents a model organism because mechanisms of gene replication, recombination, metabolism, and cell division are highly conserved in between yeast and larger eukaryotes such as human. Saccharomyces cerevisiae is budding yeast and the simplest eukaryotic organism for understanding the genetic manipulations and basic cellular processes [1]. Initially, the genetic analysis was done by mating-switch type, tetrad analysis, and gene mutation (deletion) methods to identify the genome location on a chromosome. The rise of genome sequencing technology removed all the barriers and sequenced the whole genome of Saccharomyces cerevisiae. SGD and NCBI (National Center for Biotechnology Information) GenBank [2] are the main sources of genome information such as the number of chromosomes, genes, contigs, base-pair length, etc. The cellular localization and protein number count gain the knowledge to understand the physiological role of proteins in the cell. PEDANT (Protein Extraction, Description and Analysis Tool) [3] is the proteome resource that provides information regarding proteins and their distribution according to size, molecular weight, and function. YEASTRACT (Yeast Search for Transcriptional Regulators And Consensus Tracking) [4] and SAGE (Serial Analysis of Gene expression) are used for transcriptome analysis. For the metabolic identification, yeast pathway database [5] consists of all metabolic pathways and reactions involved in the metabolism of a yeast cell. This study specifically investigated for the alcohol dehydrogenase gene ADH1 gene related genomic resources. Every kind of probable resource is utilized to find out the response related to the fermentation and the gene-responsible i.e. chromosome number, position, location, SNP identification, genes involved in the cell cycle, protein domain analysis, and distribution of proteins on different parameters with other domains of S. cerevisiae.

Evolution of Saccharomyces cerevisiae genetics

In the year 1875, Carlsberg laboratory was founded by a Danish brewer to introduce science in the brewing industry. A

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Danish mycologist, Emil Christian Hansen isolates the pure single colonies of yeast in Carlsberg laboratory and propagates them as a single strain. After that, brewery starts using a single strain of yeast name given as *Saccharomyces carlsbergensis*[6]. After that, the pioneered research on genetics was started by Danish geneticist Øjvind Winge [7]. He started his work with Carl Lindegren on yeast as an experimental organism. Lindegren did genetic crosses of strain EM93 to generate multiple generations that could help researchers to isolate specific trait and to characterize gene encode for that trait [8]. Winge's strains were homothallic at all and crosses are difficult, but Lingergen's strain EM93 (which was isolated by Emil Mrak) is heterothallic and easy to construct crosses [9]. Then, the final strain Saccharomyces cerevisiae S288C was developed by direct and intervening crosses between EM93, EM126, NRRL-210, EM93-1C, EM93-3B, 1426, 1428, CxL-10, 62, 67, 99R, 276/3BR, 463-2d, 1941,1198-lb, and S177A [10]. Due to non-flocculent conditions and minimal nutrient requirements for this strain, researchers used to deal with varying levels of congenicity through backcrossing with the parental strain, until molecular tools were not available [11].

Saccharomyces cerevisiae is used for analyzing similar cellular structures and life cycles with multi-cellular eukaryotes like animals and plants. Yeasts are non-motile, non-pathogenic and can easily propagate and manipulated [12]. The eukaryotic micro-organism observed with a nucleus and membrane-bounded organelles such as peroxisomes, endoplasmic reticulum, Golgi apparatus, and mitochondria. Yeast explored the mechanism and complex processes like cell division, energy metabolism, chromosome replication, transcription, translation, subcellular structure and function, and intracellular signaling that all are carried out by other eukaryotes [13]. Phenotypic characteristics were identified by the approach of deletion or disruption of gene loci to find the cellular function of a gene [14]. Subsequently, metabolic gene mutation became the selectable genetic marker indication to differentiate strain's genotype. The important point to study is the homologous recombination in which the DNA template used as a substrate for repair. Yeast has this characteristic to repair DNA damage and to segregate homologous chromosomes at the time of meiosis. So, research on yeast helps to learn about fundamental knowledge of other eukaryotic organisms [12]. The genetic nomenclature of genes of Saccharomyces cerevisiae was described by three letters and numbers next to it. For example, the histidine biosynthesis gene is written as HZS3 in italic format. Dominant alleles are in capital letters as CAN1 and recessive alleles are written in lower case as ura3 [13]. The gene deletion method was used to cause mutation and then identification of the gene and its protein expression level (for example, if the ADH1 gene for the alcohol production is mutated, then the acetaldehyde production increase during the anaerobic fermentation process). Similarly, if gene encoded for toxicity resistant is deleted, then the resulted mutant becomes sensitive to that toxic compound. So, these are the strategies used for the identification of protein-coding genes. However, this is not applicable when a mutation does not relate to the protein expression or the genes responsible for the metabolism.

Mating-Type Switching

It is a programmed DNA-rearrangement process that controls cell differentiation in yeast. *Saccharomyces cerevisiae* has three types of cell categories a, α, and a/α. The DNA content of each cell type differs at the genetic locus called MAT (Mating Type). These haploid types of cells possess two silent and one active copy of mating-type locus. These cells can reversibly interconvert by the process known as mating-type switching. This differentiation must be reversible because each cell must have the ability to produce each type of cell. During mating, a cell becomes α cell because it changes its genotype from MATa to MATα at the mating locus [15].The sexual reproduction in yeast undergoes by mating self-sterile (heterothallic) or self-fertile (homothallic) conditions. Switching with its mating type and mating with its daughter cells causes homothallism. The HO gene represents homothallic behavior and HO mutants show heterothallism. HO encodes a site-specific nuclease that describes mating-type switching [16]. In the middle part of the right-side arm of chromosome III has this MAT locus. Both MAT a, α is placed distinctly nearby 700 base pairs from each other as Ya and Yα, respectively. Ya and Yα regulate sexual activity as they contain promoters and open reading frames. MATα encodes MATα1 and MATα2 proteins, which express protein Mcm1 constitutively and activate the genes encoding for mating pheromone. Similarly, theste2 recessive gene encodes a transmembrane receptor of the opposite pheromone MATa [17]. The mating-type switching is a recombination approach to study cell differentiation, cell division. In starving conditions, mating generates tetrad and these help in the identification of locus at centromere through crossover analysis. This mating type switch analysis was also proposed for the selection of the trait for ethanol tolerance [18].

Tetrad analysis

During the process of mating, cell of opposite type stimulates fertilization. Cells fused to form diploid. When newly formed zygote divides mitotically produces diploid colonies but when undergoes meiosis generates 4 haploids (2:2 segregation of a andα). Haploid spores are generated when diploids are starved of nutrients. These four spores finally form a tetrad or ascus surrounded by a thick wall and become resistant to the environment. In tetrad analysis, the haploid colony is separated by microdissection on solid media and replicas are plated on different media. When a mutant is crossed with wild-type, phenotype shows 2:2 segregation (mutant phenotype is caused by a single locus). If there are

two markers then segregation resulted in three types: parental, non-parental and tetrad. The relative frequency indicates genetic linkages. When markers are far from centromere then tetrad frequency is higher. While, when markers are very close to the centromere, crossover between marker and centromere arise tetrad. Tetrad analysis revealed the genetic position of the centromere to generate maps [12]. The ascospores generated through tetrad can be used to analyze the phenotype (due to mutation). If there is a mutation then the frequency and crossover differ, this helps in identifying the position of the gene on the chromosome.

Evolution of Saccharomyces cerevisiae genomics

The complete set of genes in a cell called Genome. The central dogma follows the information encoded by DNA, which is well-organized into genes that are transcribed into RNA and translated into proteins. Molecular biology involves sequencing the DNA and to understand the genetic code, which expresses in the form of biological function [19]. The comprehensive map of the cell includes cell structure, function and expression, genome sequence, gene expression, protein localization, and post-translational modifications. Genomic information is the basic platform to study the evolution of cellular structures of human [20].From the first update of Saccharomyces cerevisiae in 1996, a new S288C 2010 genome version was revised and found the number of codon sequences was changed [21]. Lindergren and Hawthrone studied the mating type, and isolate Saccharomyces strain S288C [22]. In the context of genome advancement, they also construct their genetic map. In 1951, Lindergren published a map of 5 chromosomes and gave them the Roman numeral, which becomes the standard representation of chromosome number. In the 1960s, Hawthrone and Mortimer presented the 10 chromosomes map [23]. Motrimor with Schild further contributes in the 1980s to 17 chromosome numbers, which was later confirmed that the 17thchromosome was left arm of the sixteenth chromosome. In 1984, Kuroiwa visualized 16 chromosomes in the meiotic nuclei through fluorescent staining. Finally, in 1992, Mortimer established that Saccharomyces cerevisiae had 16 chromosomes in its 11th edition of publication [24]. Simultaneously, In the late 1980s, sequencing of yeast chromosome started with chromosome III of 315 kb sequence with 182 ORFs for proteins, which was completed in 1992 [25]. In 1996, Saccharomyces cerevisiae became the first eukaryotic organism for complete genome availability. A 16 chromosomal genome sequence with 12,068 kb size was revealed, which defines 5885 genes encoded for protein, 140 genes for rRNA, 40 for snRNA and 275 for tRNA. This was the main milestone of eukaryotic genome research [26]. The international effort of 600 scientists from more than a hundred laboratories of Europe, the US, Canada, and Japan has resulted in a 12 million base sequence. For this, around 300,000 independent sequence reads were assembled to the final sequence with less than 0.03% error rate [27]. After that, the genomic revolution of sequencing other eukaryotes was started.

Yeast Genomics resources

SGD (Saccharomyces Genome Database) is the most common platform for providing Saccharomyces cerevisiae information [28]. It provides information regarding its gene, encoded features, variants, etc. Whole genomic data is integrated into various tools. It maintains chromosomal sequence references and data sets. SGD has also submitted Saccharomyces cerevisiae S288C chromosomes information to the NCBI reference genome [29]. In the NCBI database (tillMarch 2018), a total of 2963 fungal whole genome sequence databases were available. Out of 2963, 2172 comes under ascomycetes in which 339 different Saccharomyces cerevisiaestrains data was present. Genbank, ENA, and DDBJ are other informative tools that have 255, 79 and 5 sequences respectively [30]. GenBank is called a comprehensive database source of NCBI (maintained by NIH) that has all publicly available nucleotide sequences. The international collaboration for data development of the nucleotide sequence is made under GenBank (NCBI), DNA Data Bank of Japan and in European Nucleotide Archive. An accession number is assigned for each sequence submitted by any means such as by direct submission, by bankit, by the portal or by tbl2asn [2].

ENA is a depository for the nucleotide sequence information which contains raw data, assembly, and annotation information. ENA classified into Sequence Read Archive (SRA) and Trace Archive [31]. The ENA provides services to access data and to provide information regarding the genomic sequence of a species. It is made up of a three-step process of data: (1) Reads (reads the data of nucleotide bases), (2) Assembly (relates overlapping sequence reads into contigs), (3) Annotation (interprets the biological function of the assembled sequence) [32]. Table 1 denotes the ENA data of the Saccharomyces cerevisiae genome sequence.

DDBJ, which is a member of INSDC (International Nucleotide Sequence Database Collaboration), is used to collect sequence data of nucleotide and provide free access for research activities. It collects, annotates and releases the data. The search and analysis service are categorized asGenentry (annotated data by accession number), ARSA (annotated data by accession number and keywords), DRA (Sequence Read Archive data by accession number and keywords), TX search (taxonomy base) and Blast (Homology base). DRA is most commonly used to search sequence data. In DRA search, there are 1152 studies found with Saccharomyces cerevisiae comprises of 130 WGS type [33]. For complex entries, the XML languages are developed to facilitate the task [34].

OriDB (http://www.oridb.org/) is the database that predicts the replication origin sites and summarizes them. The origin is proposed for collated and curated data sets limited to only two species. One is budding yeast Saccharomyces cerevisiae and the other is Saccharomyces pombe and it can be found at websites http://cerevisiae.oridb.org/ and http://pombe.oridb.org/ respectively [35]. Each origin site has been given a status category in the form of confirmed or likely or dubious status. Each status depends on the accuracy of the site of origin. There are a total of 829 origins of databases, of which 410 are confirmed, 216 are likely and 203 are dubious as shown in Table 2 [36].

Table 1: ENA data of Saccharomyces cerevisiae genome sequence

Assembly	Sequence	Coding	Non-coding	Read	Study	Sample	Submission
62	10,892	8011	285	4476	558	732	19

Table 2: Chromosome-wise origin of a database of Saccharomyces cerevisiae.

Chromosome Number	Confirmed origins	Likely origins	Dubious origins	All origins
I	14	7	0	21
II	37	9	17	63
III	21	2	9	32
IV	51	20	22	93
V	22	6	8	36
VI	17	4	2	23
VII	30	21	14	65
VIII	21	13	9	43
IX	15	12	11	38
X	29	7	8	44
XI	21	10	13	44
XII	32	6	33	71
XIII	27	17	14	58
XIV	21	19	14	54
XV	27	32	11	70
XVI	25	31	18	74
TOTAL	410	216	203	829

Yeast Proteomics resources

Protein is the translated product of mRNA, which directly influenced by the expression of a gene. The optimum level of protein is necessary to understand the function of a gene. The proteomic analysis is governed by the quantification of mRNA; done by microarray technology. The estimated amount of protein in 1996 was 5885 [26] and continuously updated and becomes 6002 in the year 2018, which directly affect the protein analysis [29]. Various methods can be used for the quantification of protein as Tandem Affinity Purification (TAP) with immunoblot (absolute protein abundance), Mass spectroscopy (protein copy number), and Green fluorescence protein (GFP) tags (protein localization). TAP was used firstly to globalize the protein expression in yeast. 2/3rd proteome was expressed in a range of 1000-5000 molecules per cell [37]. In silico analysis is also a good approach for proteomic analysis. It is done by information gathered for signal peptide identification, post-translational modification, and domain structural analysis [38]. These all approaches yield information to understand the complex biochemistry of the yeast cellular system. Yeast proteomes localization is done by topoisomerase I-mediated cloning and transposon mutagenesis strategies, which determines around 2744 localized proteins. Out of them, 47% are cytoplasmic,

13% mitochondrial, 13% of ER and secretory vesicles, and 27% nuclear or nucleolar [39]. Another highthroughput technique SRM (selected reaction monitoring) has been used to detect the proteins that not detected by classical methods. It measures the network of proteins with the entire translation process [40].

UniProt is a large source of protein sequence database which contains over 60 million sequences. There is a pan proteome sequence which concludes all diversity of sequences for every species [36]. UniProt resources are available on the website http://www.uniprot.org/. UniProt derived proteome information of Saccharomyces cerevisiae isgiven in Table 3. The structural and functional annotation of protein and genome occurs by SUPERFAMILY database resource. These annotations are based on the hidden Markov model, which annotated against the 2478 sequenced genome [41]. Table 4 discussed the superfamily statistics of protein in the form of sequences, domains, and alignment and domain combinations. The functional analysis of proteins and their classification into families is done by an open resource InterPro. Some predictive models described as signatures are used for generating the class of proteins. It also helps in annotations of UniProt protein sequences [42]. These are sub-categorized as all results (181) - Family (111), Domain (53), Homologous superfamily (10), Repeat (1),Sites (6) is freely available to download from EMBl_EBI site [43]. The categorization of the protein according to their size, weight, the function is needed to identify the specific nature of an organelle and localization of protein for its role. This categorization can be easily identified by the tool PEDANT (Protein Extraction, Description and Analysis Tool) is a genome and proteome database, which provides 3000 genomes with around 4.5 million proteins [44]. All sequences are covered from NCBI and it utilizes the pre-calculated information stored in SIMAP (similar matrix of protein) database. It is freely accessible at http://pedant.helmholtz-muenchen.de/ [3]. The number of proteins and their percentage is categorized based on length, molecular weight, isoelectric point, structure, and function shown in Table 5, 6,7,8, and 9 [3].

To compare the protein level difference on the base of the gene responsible, SGD Variant viewer is used. It is shown in Fig. 1 that the ADH1 gene of Saccharomyces cerevisiae compared with 11 other strains and ADH1 gene protein domains are placed from 1 to 349 base pairs. At 128, 139, and 142 position variations are shown in Fig. 1 [45].

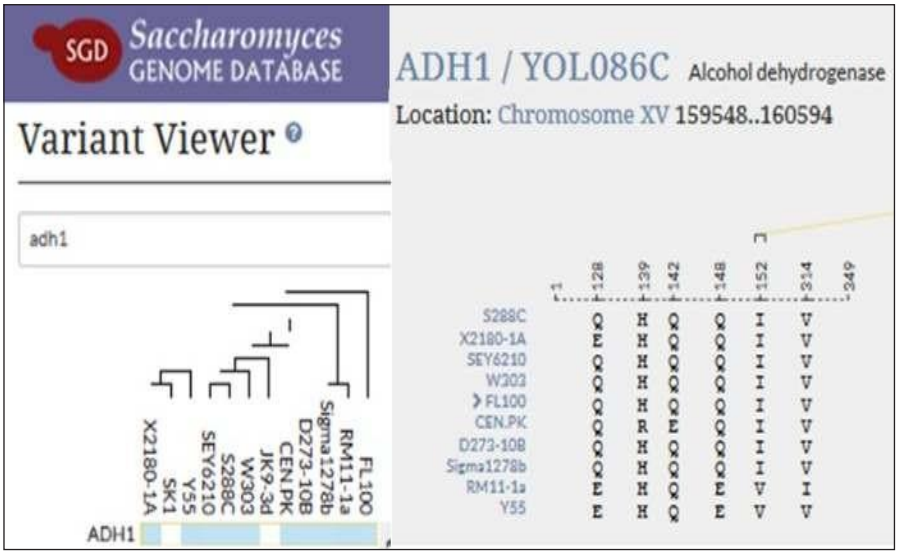

Fig.1: SGS variant viewer for identification of protein domains of ADH1 gene.

Table 3: Proteome information of *Saccharomyces cerevisiae* by UniProt

Proteome ID	Organism	Protein count	Proteome component	Genome assembly ID
Up000002311 Mitochondrion	Saccharomyces cerevisiae S288c GCA_000146045.2	6049	Chr. I to XVI; Plasmid;	

Table 4: Superfamily assignment statistics of protein in *Saccharomyces cerevisiae.*

Sequences	**6717**	**total**
	3668	(55 %) with assignment
	450	average length
	39	% amino acid coverage
Domains	**5404**	total
	778	unique superfamilies
	666	unique families
	319	average length
	6.9	average superfamily size
	86	% formed by duplication
Domain combinations	**536**	domain pairs
	1632	unique architectures

Table 5: Protein Length Distribution in *Saccharomyces cerevisiae.*

No. of Proteins	**% of Proteins**	**Length**
68	1.2%	Proteins < 50 aa
662	11.2%	Proteins 50-149 aa
1296	21.9%	Proteins 150-299 aa
3358	56.9%	Proteins 300-999 aa
515	8.7%	Proteins 1000-3000 aa
7	0.1%	Proteins > 3000 aa

Table 6: Protein Molecular weight Distribution in *Saccharomyces cerevisiae*.

No. of Proteins	**% of Proteins**	**Molecular weight**
48	0.8%	Proteins≤5.0E3 Da
263	4.5%	Proteins≤1.0E4 Da
940	15.9%	Proteins≤2.0E4 Da
3257	55.1%	Proteins≤5.0E4 Da
5194	87.9%	Proteins≤1.0E5 Da
712	12.1%	Proteins>1.0E5 Da
74	1.3%	Proteins>2.0E5 Da
1	0.0%	Proteins>5.0E5 Da

Table 7: Protein Isoelectric point Distribution in *Saccharomyces cerevisiae.*

No. of Proteins	% of Proteins	Isoelectric Point	Acidic/Neutral/Base
1	0.0%	Proteins pI < 3.5	very strong acidic
181	3.1%	Proteins 3.5 pI < 4.5	strong acidic
1286	21.8%	Proteins 4.5≤pI <5.5	Acidic
1279	21.7%	Proteins 5.5≤pI <6.5	weak acidic
535	9.1%	Proteins 6.5≤pI <7.5	Neutral
645	10.9%	Proteins 7.5≤pI <8.5	weak basic
1244	21.1%	Proteins 8.5≤pI <9.5	Basic
604	10.2%	Proteins 9.5≤pI <10.5	strong basic
131	2.2%	Proteins pI≥10.5	very strong basic

Table 8. Proteins distribution according to function in *Saccharomyces cerevisiae.*

Sr. No.	Function	Protein	Examples
1.	Metabolism	1997	Amino acid, polysaccharide, lipid, fatty acid, prosthetic groups, cofactors, vitamins, and the extracellular metabolism
2	Metabolic Energy	479	Glycolysis, gluconeogenesis, TCA cycle, ETC, Fermentation, fatty acid oxidation, etc.
3	DNA processing and Cell cycle	1333	Synthesis of DNA and its replication and recombination with Repair mechanism and control of mitotic and meiotic cell cycle.
4	Transcription	1229	RNA synthesis, transcription activities, and mRNA modification
5	Protein processing	546	Ribosome biogenesis, translation, translational control
6	Protein fate	1416	Folding of protein, its stabilization, translocation, and transport.
7	Cellular communication / signal transduction	507	Cellular signaling, JAK-STAT cascade, Enzyme and second messenger mediated signaling, hormonal induced signaling, signaling through G protein.
8	Cell rescue and defense	915	Stress response, heat shock response, antibiotic resistance, chemical agent resistance
9	Environmental response	536	Homeostasis, cell movement, cell-cell contact, osmo-sensing, and response
10	Sensing the environment	5	Response to the effect of the environment
11	Transposable elements	26	Transposon movement
12	Cell Fate	512	Directional cell growth, growth regulators, cytokines, cell death
13	Development	103	Mating (fertilization)
14	Biogenesis of cell components	1290	Cell wall, cytoplasm, plasma membrane, endoplasmic reticulum, Golgi, nuclear membrane, peroxisome
15	Cell type differentiation	585	Budding,
16	Storage protein	1	Storage facilitating proteins
17	Protein with binding function	2448	Protein, nucleic acid, polysaccharides, metal and lipid bindings.
18	Metabolic adjustments	545	Enzymatic activity regulation, ion channels, a regulator of the transcription factor, a regulator of receptor activity, transport facilities

Table 9: Structure-based protein distribution in *Saccharomyces cerevisiae.*

Sr. No.	Structure type	Proteins
1	Coiled-coil regions	1272
2	Disordered regions	5338
3	Low-complexity regions	441
4	alpha structure	2224
5	beta structure	256
6	alpha/beta structure	3261
7	irregular structure	150
8	alpha structure domains	264
9	beta structure domains	181
10	alpha/beta structure domains	822
11	α and β (α+β) structure domains	487
12	Multi-domain (α and β)	55
13	Membrane and cell surface proteins and peptides	41
14	Small proteins	42

Yeast Transcriptomics resources

The analysis of gene expression through RNA is known as transcriptomics. Various techniques carry high throughput investigation of the mRNA expression, for example, SAGE, cDNA-AFLP and Differential Display (DD), SSH (suppression subtractive hybridization), and Microarray. These techniques generate a gene expression profile in a model organism with a completely sequenced genome [46]. RNA Sequencing is the new method to sequence RNA that allows transcriptome analysis at the base-pair level. RNA library preparation and qPCR analysis are used to validate the selection of genes. Library preparation also gives additional information about the transcription start site and its structure [47]. There is a total of 424 RNA genes are present in yeast out of which 27 are rRNA, 299 tRNA and 98 are non-coding RNAs found in the cell [3]. YEASTRACT (Yeast Search for Transcriptional Regulators And Consensus Tracking) is a resource for database, which contains around 16300 regulatory associations that depend on transcription factors. There are 268 specific binding sites for DNA encodes for 118 TFs. It maintains 5995 Gene ontology terms obtained from GO terms. Promotor and nucleotide sequences can be taken from SGD. It also has a DISCOVERER tool, which is used to identify the complex motifs. YEASTRACT derive the potential transcriptional regulator of the concerned gene [4]. SCEPTRANS (Saccharomyces Cerevisiae Periodic Transcription Server) is used to analyze the microarray data related to expression in the yeast. It allows finding multiple gene profiles in any number of data sets. It also used to search co-expressed genes, correlation analysis, functional annotations and localization of data on a set of genes [48]. Yeast cell cycle analysis project (YCCAP) is an analysis tool to identify all genes in the cell cycle which regulates their mRNA levels. It has an index of the transcription change in the cell cycle process [49]. A cell cycle gene ASH1 (Asymmetric Synthesis of HO1) shown in Fig. 2 as an example in which it is placed in between the M and G1 compared with reference genes found in cell cycle processing. It also plots a graph divided into 4 experimental parts alpha-factor, cdc15, cdc28, Elutriation and a peak to trough ratio with some score [50]. For ADH1, the ratio is 0.094 which suggests that this gene is not responsible in the cell cycle, while for ASH1 peak score is 11.8 is shown as M/G1. A comprehensive transcriptome analysis tool is used to analyze the bulk RNA sequence dataset called AltAnalyze. It can access exon expression with proteins with microRNA targeting. It runs through a graphical or command line. A new isoform viewer in this visualizes the protein, domains, and microRNA binding sites [51]. SAGE analysis is another method for gene expression. The methodology includes in SAGE is described as the short transcript of 10-14 bp is a tag at a unique position and joined one by one by to form a long molecule for cloning. The expression level is checked by quantifying the appearance of the tag several times [52]. SAGE analyzes the 60,633 transcripts of yeast and revealed 4,665 genes, out of which 1981 had known function [53]. SAGE transcriptome of Saccharomyces cerevisiae has 40 highly expressed genes and 30 putative coding sequences [53].

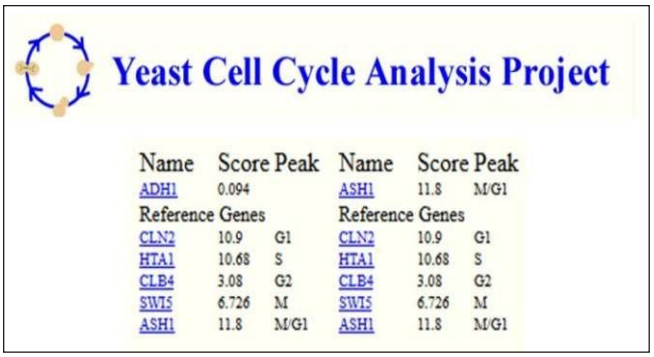

Fig. 2: Score and peak of ADH1 and ASH1 in the cell cycle of *Saccharomycescerevisiae*.

Yeast Metabolomics resources

Metabolomics is the field to identify cellular metabolites and their roles in metabolism. Metabolite works as a signaled cell product and analyzes by various analytical approaches like LC-MS and NMR. There are multiple techniques required for extraction, quantification, detection, and identification of metabolites. Secondary metabolites are more important for a defense mechanism, while primary metabolites are used in cellular metabolism. These metabolites play a role in the differentiation of species for environmental factors and considered as taxonomical markers. Direct infusion electrospray mass spectrometry (DiMS) is mostly used to classify fungal species based on their mass profile of chemicals released as secondary metabolites [54].

The metabolic network is the connection of the release of a chemical entity that can be easily used by the organism like an accumulation of amino acids. Enzymes are coded by genes and change in these genes alters the function of the whole metabolic network. The structural and functional analysis of metabolic networks can be done by various experiments e.g. enzyme activity, enzyme concentration, rate measurements from the substrate to product, kinetic study, etc [55]. Primary and secondary metabolites are produced by micro-organisms during their growth and environmental stresses. These metabolites are classified as Extracellular (releasing outside the cells) and Intracellular (inside the cells). Extracellular metabolites like antibiotics can easily be determined as compared to intracellular metabolites. Solid-phase extraction with sorbent (like silica, carbon-based sorbents, polymeric materials) is used for metabolites separation [56]. Intercellular metabolites are rapidly metabolized by the action of enzymes. The process starts with quenching, a method to arrest the cell metabolism at a stage which is done by the perchloric acid, cold methanol, and cold glycerol saline solution. After quenching, the intercellular component was extracted by boiling with water, ethanol, buffered methanol, water and chloroform ratio, or with acidic/alkaline solutions [57]. Then, these can be analyzed by the analytical techniques. The metabolic reconstruction at genome-scale and in-silico identifications have found 733 metabolites and 1175 metabolic reaction in Saccharomyces cerevisiae. Here, reconstruction means the use of genomic (identification of ORFs), biochemical (enzymes) and physiochemical (pathways) information to construct the metabolic network. It is a constraint-based approach to compute the number of ATP molecules generated in the electron transport system. mRNA based profiling, knockout gene effect, and computation of integrated functions observed 70-80% phenotypic similarity. In total 1175 reactions: 702 are cytosolic; 349 are transport-related; 124 are mitochondrial. Where, 584 are unique metabolites divided into the cytoplasm (559), mitochondria (164) and extracellular (121) regions. In silico analysis done by flux balance analysis and optimizing the constraints by LINDO (Linear INteractive and Discrete Optimizer) package [58] help identify intracellular metabolites.

Yeast Pathways Database (YPD) is the tool of SGD to analyze and visualize the metabolic pathways of Saccharomyces cerevisiae. This software is built at SRI International by Peter Karp by manual curing of pathways collected from YeastCyc [59]. Cellular overview comprises all metabolic pathways that occur in the yeast cell. The specific pathway can be quickly searched after the typing pathway name as shown in Fig. 3 extracted for glucose fermentation [5].WikiPathways is an open platform for curing biological pathways started with genes and protein products, but now it can be also used for metabolic pathways [60]. There were 20 pathways found related to yeast, which includes sugar metabolism, glycolysis, fermentation, pentose pathway, glycogen catabolism, starch biosynthesis, lactose degradation, glycerol catabolism, etc. [61].To identify the biological ontology or pathways to tell about a specific set of metabolites, the GO (Gene Ontology) Elite Pathway is used. There are multiple options for pathway visualization. It is built to provide information near 60 species covering phenotype, gene, multiple pathways and micro-RNA targets [62]. PathVisio is free software for pathway analysis. It is opened to edit, draw, and analyze the biological pathway. It became simple through a pluginthat helps in pathway analysis building pathways, analyze, and data visualization [63]. KEGG Pathway represents the reactions related tometabolisms like carbohydrate, energy, lipid, amino acid, nucleotide, and other cellular responses. It also contains vitamin, cofactor, terpenoids, or various other secondary metabolite pathways [64]. There are 116 pathways for Saccharomyces cerevisiae and 6 pathways relateto gene ADH1 and some other genes (ADH2, ADH3, ADH4, and ADH5) that also contribute to metabolism. BiGG Model is a genome-scale metabolic model that contains 75 manually curated high-quality models. It also connects the genome-scale to genome annotations. It standardizes the reaction and metabolites across models that enable rapid comparisons [65]. For Saccharomyces cerevisiae, there are 1226 metabolites, 1577 reactions and 905 genes responsible that are present with BiGG ID iMM904. Small Molecule

Pathway Database is designed for metabolism elucidations and it is used in system biology [66]. It gives an overview of the pathway, diagrammatic process, metabolite location, cofactors, chemical structures, etc. For Saccharomyces cerevisiae, there are 9549 metabolite results and 4 signaling pathways related to stress. Reactome tool gives 3 metabolic pathway data of Saccharomyces cerevisiae and 48 kinds of reactions [67]. MassBankis the repository of a small compound mass spectra dataset. There are EI-MS (605), ESI-MS (9276) data of 2337 metabolites and fast atom bombardment MS (137) data available [68]. MassBank is useful for structural and chemical identification of these metabolites detected by MS.

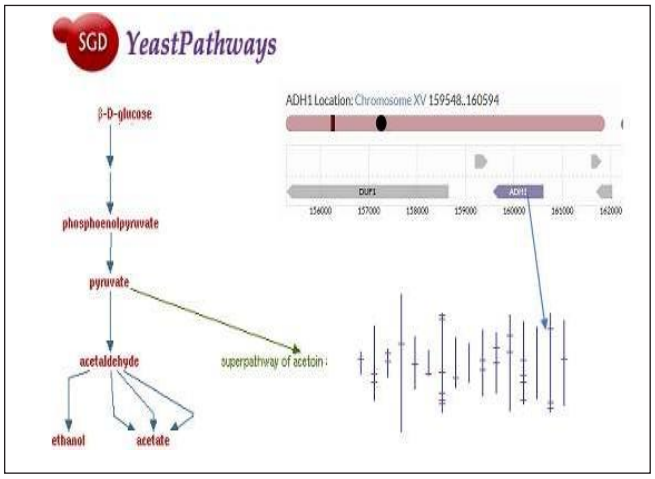

Fig. 3: Glucose Fermentation in *S. cerevisiae* and ADH1 gene position on chromosome XV.

Yeast SNP database

SGD Saccharomyces Genome Database is the reference genome resource specifically for yeast (https://www.yeastgenome.org/). SGD provides correlation and comparative analysis of variation in phenotypes. A variant viewer of SGD shows the variation in their DNA and protein but not available on NCBI or Yeast Resource Center Snip Viz [45]. Variation in aligned sequences occurs by SNPs, insertion, and deletion. Different types of SNPs synonymous, non-synonymous, intronic can be visualized on the map of the specific chromosome. Alcohol dehydrogenase is the key enzyme plays a specific role in the fermentation and encoded by gene ADH1 present on the chromosome XV. In the variant viewer, a total of 12 different strains of the yeast can be compared for this specific gene. It results in the SNPs location and type on the chromosome (shown in Fig. 4). There are around 60,000 SNPs and 6000 insertions or deletions found in between S288C and YJM789 strain [69, 70].

Yeast biological system modeling

Saccharomyces cerevisiae is used as a cell factory for the production of various products. It is commonly used for alcohol production, lactate, pharmaceuticals, insulin, and

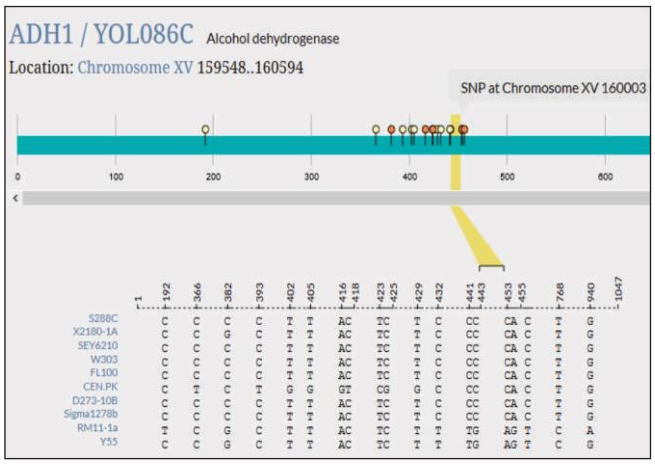

Fig. 4: SNP variation in different yeast strain on a single ADH1 gene on the Variant viewer.

vaccines [71]. In the year 2014, Boone was focusing on the global mapping of interaction networks and invented SGA (Synthetic genetic array) technology. It gives automation to cross multiplenumbers of strains carrying mutations [72]. System modeling is featured by system biology. System biology is the combination of novel techniques from different fields like functional genomics, mathematical modeling and bioinformatics [73]. There are various tools used in system biology to determine the quantitative description of the biological system [74].

Bioperl tool is open to all users in the association of genomics, bioinformatics and life sciences. It was also part of the human genome project [75]. It provides software modules for programming. The process continues as accessing nucleotide and protein sequences, transforming into formats, manipulates sequences, then creating sequence alignments and then search for other structures and finally annotate them [76]. Biopython is an open-source bioinformatics tool written in python language. It includes various modules of writing, reading formats, multiple sequence 49. alignments, interactions with BLAST, ClustalW and EMBOSS and provides numerical methods for statistics [77]. Cytoscape is another open platform for identifying molecular interaction networks like DNA-protein and protein-protein interactions with biological pathways and joining these networks with annotations, expression profiles, etc [78]. There are more tools are designed as information resources for system modeling. COPASI is used for the analysis of biochemical networks by simulation methods [79] while Jcell is a Java-based software work for the reconstruction of genetic interactions from microarray data [80]. Sys-bio also enables reliable predictive models of biological systems at the cellular level. SBOL (system biology open language) and SBML (system biology markup language) are part of Sys-bio [81].

Gene function technology platforms

It is an approach that provides a systematic function of the genome sequence. The single gene determination with heritable diseases in yeast is around 40% and is found homologous [82]. There is a report was announced in 2002 with the completion ofthe S. cerevisiae gene deletion project, that 18.7% of total 5916 ORFs are essential for growth function [83]. Functional analysis is based on the genome, transcript, proteome, and metabolic platforms discussed below.

Genome-level: In the genome level process, a single ORF deletion can be done by the PCR method [84]. If there is a change in phenotype that can easily be marked out. During the yeast deletion worldwide program, a part of the oligonucleotide is used for that replacement and the molecular barcode is given for each deletion in mu tant [85]. So, if a part of toxin resistance removed, it made the cell to be sensitive to that drug and helps in identifying the target.

Proteome level: Proteins are functional elements of the cell; those can be encoded by single or multiple genes due to different splicing and post-translational modifications. 2D gel electrophoresis with peptide mass fingerprinting analyze the proteome [86] but does well for an amino acid or near-neutral isoelectric points. Ion-exchange chromatography improves the purification of protein complexes through tags [86]. Yeast 2-hybrid system is used to observe the interactions of proteins between homo and heterologous proteins in the yeast [87].

Transcriptome level: The hybridization-array technology is used to analyze the expression of a gene which is developed by the functional region within the genome that converts it into protein. The examination of the expression of a small number of genes can be done by Northern hybridization. Apart from that, a comparison was done by [88] with 1003 yeast ORFs and pointed out comparative expression data. So that it emphasizes to normalize the raw transcript data, those are overlapping at different places.

Metabolome level: Many yeast deletionsdo not show any phenotypic effect, but it affects the growth rate fluxes and intracellular concentration of metabolites. So that metabolite analysis reveals the activeness of that protein and its site of action. This can be resolved by constructing a systematic database of response indicator profiles carries single ORFs deletions in any gene type. The database can be screened in between known and unknown metabolic profiles to find similarities or differences with the involvement of any novel gene [89].

Potential Utilization of yeast genomic and proteomic resources

Saccharomyces cerevisiae has a short generation time, which makes it useful for industrial use [90]. It is used to find the function of the gene because it is easy to do manipulation in its genome and facilitating the function related to humans [91] whereas it is difficult at the phenotypic level. There are some targets and applications discussed here for resource utilization in different fields.

Drug targets: Yeast cell and tumor cell growth is inhibited by several cancer drugs by the same mechanism as found in human therapeutic targets and opens the way to develop and screen new chemical compound which is medically relevant [92].

Protein expression analysis: The specific protein identification,in-silico analysis, andpost-translational modification study help to identify the role of specific protein and their role in metabolism. mRNA and transcription factors allow us to find the response related to the exonic region-specific results and it differentiates the expressive part of the genome sequences. The cellulase enzyme production in *S. cerevisiae* for efficient ethanol production requires these resources to be utilized for a potential target of specific genes involved and how they are expressed in other systems. The bgl1 gene from Trichoderma sp. to yeast requires its expression on the cell surface and should be extracellular.

Fermentation metabolism: The metabolic pathway analysis, enzymatic reaction, and transporters help to find the intracellular and extracellular responses of the cell. The metabolic secretion among species is different and correlates with the genome. From the industrial point of view, this helps in the enhancement of productivity through metabolic pathway changes. Prior knowledge of the genes like alcohol dehydrogenase gene ADH1, their location in the chromosome, pathway-related with it and reactions involved, helps in further advancement in the strain at the genetic level and relates with them in expression and metabolism. Genetic improvement increasesfermentation efficiency. Overexpression of the ADH1gene is a suitable target for further improvement.The metabolic engineering approach enhances the yield of ethanol from pentose and hexose sugar from lingo-cellulosic biomass. Theheterologous genes XYL1 and XYL2 found in Pichia stipitiswere expressed for the xylose consumption for xylose to xylulose and xylulose to xylulose-5-phosphate respectively, which further contribute PEP (Pentose Phosphate) pathway for ethanol production [93]. These genes may help is developing co-fermenting yeast.

Industrial areas: Low cost of culture media, fermentation ability, fewer byproduct formations make it advantageous to be used for production. Nowadays, yeast is used for the production of biofuels in which conversion of plant polysaccharide to ethanol is done. Genomic analysis and insertion of pentose fermenting genes help in further

improvement of the ethanol production from lignocellulosic biomass.

Pharmaceutical /Therapeutics: Yeast is also used to produce proteins because complex secretory polysaccharides are immunogenic. Hepatitis B vaccine is the first genetically engineered [94] vaccine prepared from yeast and particles can be easily harvested from the cytoplasm [95]. It can also be used for the production of secreted proteins or antibodies. The first recombinant protein was human interferon, which was produced in 1981 by yeast *S. cerevisiae* [96]. The heterologous protein [97] production is also highlighted in yeast as a pro-insulin precursor of insulin is also produced by *S. cerevisiae* through protein engineering [98]. Thus, *S. cerevisiae* has a wide range of utilization in a different field related to the advancement through biological systems.

Summary

The first widely used model eukaryotic organism sequenced is Saccharomyces cerevisiae. The genome identification of the yeast describes the characteristic feature of the species related to humans or other higher eukaryotes. Its cellular metabolism response, protein expression, RNA, and DNA information help the researchers to study different approaches. Variousdatabase resources can explore information with the use of computational tools. SGD, PEDANT, YPD, and NCBI are the open platform for gathering maximum information regarding all resources. The utilization of these resources can be helpful in the development of yeast to generate a modified strain that becomes valuable and industrially important.

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Conflict of Interest:

The authors declare that they have no conflict of interest.

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ALGAE DERIVED HIGH-VALUE GREEN BIOPRODUCTS

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Abstract
Microalgae are a renewable potential source of high-value products. The superficial activity of CO_2 sequestration and accumulation of lipids inside make it useful for large scale cultivation. Constant high energy demands and depletion of fossil fuel resources have focused on the technologies to improve biofuel yield through micro-algae development. The ability of microalgae to producehigh-value products, such as DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) helps in increasing their global demand. This review focused on production, purification of DHA, EPA and carotenoids, their operational conditions, bio-synthesis pathways, and applications.

Keywords
DHA, EPA, Carotenoids, Downstream Processing, Applications.

Introduction

A photosynthetic organism with short doubling time and faster growth, mostly found in fresh and marine water is known as algae [1]. The ability to fix carbon dioxide and to store fatty acids in the form of lipids makes it very beneficial to produce different valuable products like DHA and EPA. The photosynthetic nature of micro-algae helps in pigment formation such as carotenoids. These pigments are high in demand and value in cost, even if produced in a low amount. Microalgae are now becoming an epic center for biofuel production due to its higher lipid accumulation ability. They also contribute to the fixation of CO2 with a very less amount of carbon footprints [2]. Microalgae can be used in various applications such as the production of biodiesel, bio-oil, biohydrogen, bioethanol, biobutanol, biomethane, pigments, vitamins, antioxidants, pharmaceuticals, fertilizers, fiber, agar, food supplements, and major high-value products like docosahexaenoic acid and eicosapentaenoic acid [1]. Micro-algae are sustainable sources to produce third-generation biofuel and gaining interest in being used as a renewable source of energy [3]. The mass cultivation of algae in controlled conditions generates the need for specially designed bioreactors. Open ponds and closed photo-bioreactors are mostly designed in such a way that photosynthetic activity should not be compromised [4]. There are a variety of photo-bioreactors, such as airlift, horizontal, flat, stirred tank, bubble column or hybrid bioreactors used and designed specifically to reduce their maintenance cost [5]. Some of the micro-algae are top in the list for DHA and EPA production, for example, Crypthecodinium cohnii, and Microtus subterraneus respectively [6]. The primary and secondary carotenoids are also concerned about their vast application in the food and pharma industries [7]. For various kinds of application and development of valuable products, microalgae are diversified into cyanobacteria, prochlorophyte, eukaryotic algae, green algae, rhodophyta, heterokont algae, prymnesiophyta, cryptophyta, extremophilic algae, and dinoflagellata[8]. This review signifies the various aspects related to production medium, product application, downstream processing, importance, and market potential of the DHA, EPA, and carotenoids purified from micro-algae.

Algal derived high-value products

Docosahexaenoic Acid

It is one of the types of omega-3 fatty acids. It is also a human brain development constituent of the cerebral cortex, skin, and retina. The main sources from where DHA is obtained are maternal milk, fish oil, and algae oil. Among the fishes, species as the main ω-3 fatty acid sources are- Herring, Mackerel, Sardine, and Salmon. Mainly, the fish oil is the

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core of ω-3 fatty acid, but oleaginous microorganisms can also have the potential for it. The microalgae have been a preferred choice for the production of ω-3 fatty acid because of following reasons: 1) High growth rate, 2) Higher biomass density, 3) No competition for land space, 4) Some of them can convert phototrophic metabolism to heterotrophic metabolism in the absence of light, 5) The proportion of ω-3 fatty acid is more than that of other lipids, and 6) Can tolerate low salt conditions [9].

Importance and applications of DHA

DHA is the key component of the plasma membrane (especially in neurons) and in tissues found in the brain, eyes, heart and cardiovascular system. Daily dietary intake of ω-3 fatty acid (DHA and EPA combined) has been estimated to 250-500 mg per day. The focus has been made to supplement the daily diet of an individual to meet the demands of daily dietary intake. Deficiency of DHA results in various disorders like lack of memory, impairment of visual, immune neural response. DHA plays following functions in the body (Huang et al): 1) maintains the blood pressure; 2) maintains normal blood triglyceride levels; 3) regulates normal brain function; 4) regulates the vision; 5) regulates the development and functioning of the brain; 6) anti-inflammatory properties; 7) improves memory, and 8) it plays some role in the regulation of cell cycle. DHA obtained from algae has some plus points over the DHA from fishes. This has solved the problem for vegetarians. As DHA from fishes has a fishy taste and is considered as non-vegetarian, vegetarians can derive their DHA supplement from algal oil. Ocean-borne contamination can also be avoided using algal DHA. Similarly, there are some side effects of DHA also as nausea, prolonged bleeding, and bruising [10].

Methods of DHA production from algae

If the product is going to be used for human consumption, both the product and source should be non-toxic and non-pathogenic. Microalgae are the primary producers, which in the absence of nutrition (algal bloom) accumulate lipid in the biomass. Microalgae prove to be a good source to produce DHA, but restriction lies in the economically feasible processes to produce DHA. That is where the role of species comes. Both photoautotrophic algae and heterotrophic algae are a good source of DHA but to produce DHA heterotrophic algae are frequently used. The problem with photoautotrophic algae is that the net content of lipids produced is low. The need is to identify species/strain that can be used commercially. Various algal members of the class Dinophyceae, Bacillariophyceae, Chlorophyceae, Prymnesiophyceae, and Euglenophyceae are known to be containing a good amount of DHA. Other organisms, like Thraustochytrium, Schizochytrium also accumulates a good amount of DHA in their biomass. Crypthecodinium cohniihas been engineered and used commercially for the large production of DHA in industries. This species is also important because it produces DHA more than other polyunsaturated fatty acids. Schizochytrium species are also used to produce DHA [11].

Production of the DHA is mainly affected by three factors: 1) Strain (which is being used), 2) Culture conditions that mainly involve media components 3) Environmental parameters. While the rate of production of DHA depends on the following things: 1) Concentration of the biomass, 2) Cell lipid content, 3) DHA content of the lipid. For every strain, conditions for the growth and production differ. Due to this difference, the production of DHA requires optimization of the process. A two-stage culture process works best for most of the organisms. In this process, first is the cell number increasing stage where all the conditions are provided for the multiplication of the organism. The second stage is the cell size increasing stage, where the size of the cell increases and the accumulation of lipids takes place [12]. Usually,fed-batch processes are used to produce DHA, but semi-continuous and continuous processes can also be used for its production. These processes must be modified to match the conditions required for the microalgae.

Production media components and conditions specific to DHA production

Different strains have different requirements. There is no universal media suitable for every strain. Basic components of media and conditions are the same for every organism such as carbon, nitrogen, dissolved oxygen, a suitable temperature, and pH. Efforts have been made to use cheaper sources of carbon and nitrogen to make the processes economically feasible. Cheap carbon sources that have been used are: Sweet sorghum juice, Coconut water, Crude glycerol and the nitrogen sources that have been used are ammonia and spent yeast [13]. The role of macronutrients and micronutrients has been discussed as the following sub-sections [14].

Carbon Sources

The use of different carbon sources affects the yield of DHA. Glucose, dextrose, ethanol, acetic acid, sodium acetate, carb pulp syrup, galactose, etc. are some of the major carbon sources used for the culture of different microalgal species. Glucose and galactose enter glycolysis and results in the formation of pyruvic acid, which later results in the formation of acetyl coenzyme A. Acetyl coenzyme A serves as the basic molecule for lipid production. The pathway for the synthesis of DHA is given in Figure 1 [15]. It starts from Malonyl-co-A in the cytosol and continues in the conversion of different kinds of fatty acids and finally formed into a 22 carbons-DHA. The most common source used for the growth of C. cohnii is glucose. When acetic acid was used, the highest DHA and highest biomass were obtained. When ethanol was used as a carbon source, the highest volumetric productivity

was obtained, while the highest quality of lipid, when the source is glucose. Starch and glucose both are the major carbon sources for the cultivation of Thraustochytrium sp.

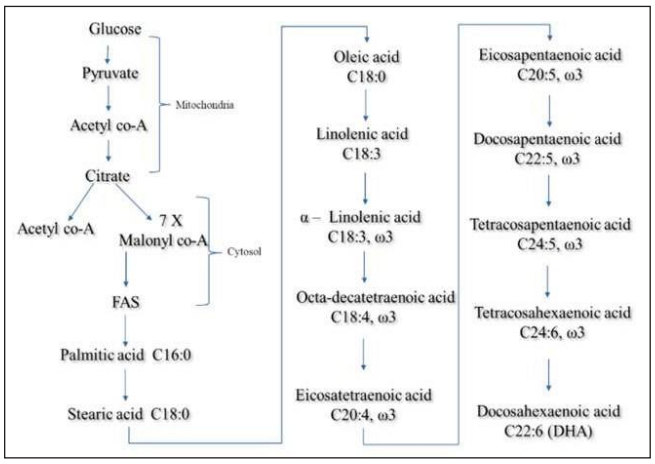

Fig. 1: Biosynthetic pathway of DHA

Nitrogen Sources

Nitrogen sources play an important role in the growth phase. Once the nitrogen sources are depleted, microalgae start accumulating lipids. The main sources of nitrogen that are being used are glutamic acid, yeast extract, peptone, meat extract, molasses waste, CSL (corn steep liquor), potassium nitrate, and ammonium chloride. For the cultivation of Thraustochytrium sp. sodium glutamate was used. Among organic and inorganic nitrogen sources, organic sources were more preferred by the organisms. Yeast extract and glutamic acid are majorly used among organic sources.

Vitamins

For high volumetric productivity of Schizochytrium sp., vitamins like thiamine, biotin, and cyanocobalamin are required. C. cohniiis an auxotroph for thiamine and biotin. But using yeast extract cost of additional vitamin requirement can be reduced, as yeast extracts cover all the contents of nutritional factors required for the growth.

Salinity

Mostly, DHA producing microalgae is marine, therefore maintaining salinity is an essential requirement. Salts are used to mimicking the saline conditions present in seawater. Sodium salts, phosphorous, magnesium, potassium salts, borates, and carbon silicates are required in the culture media. Some salts are needed in trace amounts like iron, manganese, cobalt, zinc, chlorine and bromine salts. Salts should be used in proper amounts as higher salt conditions cause corrosion in the fermenter. The growth of C. cohniiwas inhibited at 0.3% and 0.5% NaCl concentration. For the phycomycetes optimum concentration of NaCl was found to be 2.5%. Care should be taken while using chlorinated salts as chorine plays a major role in corrosion. To avoid corrosion, a non-chloride salt-sodium sulfate was used for the large-scale cultivation of Schizochytrium sp.[9].

Dissolved oxygen and pH

Dissolved oxygen is needed in the growth phase of the microalgae whereas, for the lipid accumulation phase, low oxygen conditions are required [16]. A pH range of 5.2-7 is the range which is suitable for most of the organisms. Proper pH should be maintained otherwise there would be stress on algal species and a lot of energy would be wasted on maintaining the pH. It had been studied that a range of 6.6-7.2 was best for the C.cohnii in shake flasks. And to produce C. cohniiat an industrial scale, the pH range of 7-7.8 was best suited.

Temperature

Optimum temperature is required for the accumulation of lipids in cells. Higher temperature favors the growth, while lower temperature favors the lipid accumulation. DHA content was improved by 20% when C. cohnii was grown at 25°C for 48 hours and then reduced to 12°C for 24 hours as compared to the culture that was grown for 72 hours at 25°C. The same kind of results was observed for Mortierella, Entomophthora, and Isochryses jalbaua.

Basal medium

Basal media in g/l required for maintaining the proper culture condition in Thraustochytrium aureum had been defined as Sodium Chloride (25), Magnesium sulfate heptahydrate (5), Potassium chloride (1), Potassium dihydrogen phosphate (0.1), Calcium carbonate (0.2), Ammonium sulfate (0.2), Sodium glutamate (2), Thiamine-HCl (10 µg), Sodium bicarbonate (0.1), Vitamin B12 (1 µg) [17]. Media composition varies with the species used to produce DHA.

Reactor types

Stirred tank reactor: Production of DHA from C. cohnii and Schizochytrium sp. incriminates the use of a stirred tank reactor (Martek Corporation). The advantages of using a stirred tank reactor involve: - 1) sufficient blending to avoid the formation of concentration gradient, 2) transfer of oxygen should be efficient. The risk of getting corroded is high; therefore, high-grade stainless steel is required to withstand corrosion. The use of glass reactors is an alternative approach but not suitable at a large scale.

Airlift bioreactor: Proper mixing and aeration needs for algal growth. Airlift reactor is suitable is this case. For the cultivation of Aurantiochytrium sp. at a pilot-scale airlift bioreactor was used. The volumetric productivity obtained was high. The reason for the increased volumetric productivity was the increase in shearing force. The use of airlift bioreactors is economic at the industrial scale but chances of contamination increase.

Cultivation strategies

Batch culture: Rosa et al. used two-staged cultivation processes for the growth of Aurantiochytrium limacium SR 21 by changing the C: N ratio. At first stage C: N ratio was

10:1 and at the second stage, the ratio was 55:1. high volumetric productivity was significantly higher.

Fed-batch cultivation: Martek Corporation has developed a two-stage process using the fed-batch process to produce DHA. In the first stage, the cell actively divides and grows exponentially in the presence of glucose and yeast extract. Once the Nitrogen source (yeast extract and peptone) starts getting depleting, the cells start accumulating DHA.

Continuous cultivation: Pleissner et al. studied the growth of *C. cohnii* in continuous flow culture to determine the effect of various nutrients on growth.

Downstream processing of DHA

There are various methods for DHA production but very few are used at large scale production. Three methods that are generally used in the downstream process of DHA are enlisted as a) urea fractionation [18]; b) solvent extraction [12]; c) supercritical fluid extraction [19]. The process of purification starts with the cell disruption by milling, shearing or by sonication, it was then separated by solvents like hexane followed by chemical refining and neutralization. Later, degumming and winterization of the crude lipids for the extraction of the purified products are completed. The process flowchart is shown in Figure 2.

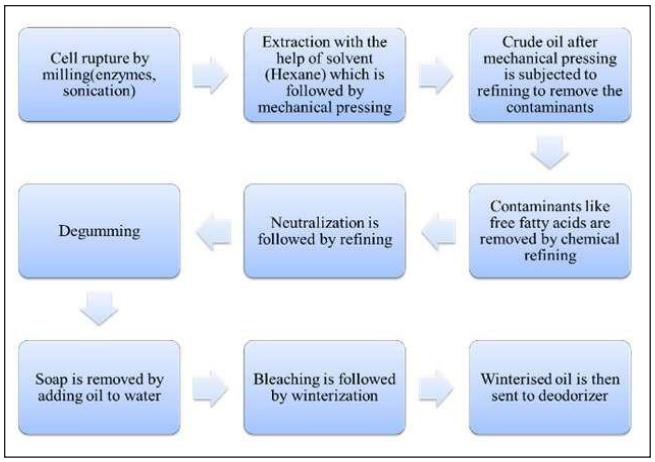

Fig. 2: Downstream processing of DHA by solvent extraction

Genetic engineering for DHA improvement

The obstacle in obtaining high productivity of PUFA is since we lack strains that have high biomass productivity and high content of PUFA. If genetically modified strains would be commercially available, we could reduce the limitation with the photobioreactors like dependency on light and low growth rate. Genetic engineering is not very successful in the case of diatoms as they lack a genetic transformation system. As the whole genome sequence of *P. tricornutum* and T. pseudonana are available, these organisms have become the putative candidates for genetic and metabolic engineering. Enzyme producing genes insertion enhances the production of PUFA. Like Δ6-elongase from T. pseudonana and Δ5-elongase, Δ4 and Δ5-desaturase from other algae was expressed in yeast and it led to the successful production of DHA. With the development of functional genomics, genetically engineering is proving a better way to improve the production and quality of PUFA in microalgae [18].

Market potential of DHA

As the demand for DHA is increasing the global market for DHA is also increasing. The advantage of micro-algal DHA over fish DHA has triggered the growth of the market of DHA. The global market of microalgal DHA includes seven regions – the AsiaPacificexcept for Japan, Western Europe, North America, Eastern Europe, Latin America, and Africa. The AsiaPacific except Japan has the highest global market for microalgal DHA. The market size of PUFA ingredients from dietary supplements could surpass 95 kilotons by 2022. ω-3 ingredient market size from dietary supplement generated over 1 trillion business in 2014. The competitors in the ω-3 ingredients market are NU-MEGA Ingredients Pvt. Ltd., FMC Corporation, Omega Protein Corporation, Croda,Inc., BASFSE, Arista Industries, Inc., Pronova Biopharma ASA, and Copeinca ASA [20-22].

Eicosapentaenoic acid

Importance of EPA

The importance of EPA has been reported in current production studies as well as in clinical research. EPA (eicosapentaenoicacid) is a major fatty acid in respect of health [23]. EPA acts as a precursor for other fatty acids and performs as a health supplement for various mediators present in the immune system [24]. EPA is an essential omega-3 polyunsaturated fatty acid that is not produced by the human being in enough quantity. The major sources for EPA are fish oil, microalgae, and some genetically modified plants. In the human body, EPA is produced by the conversion of alpha-linolenic acid but not in sufficient quantity, because of this, the actual requirement of the body not gets accomplished. So, the rest need for EPA can be fulfilled through diet.

The production of EPA from marine fish is very low and recovery is also expensive. These drawbacks lead to the production of EPA from algae. Most of the studies focused on the production of EPA from marine fishes. The production of EPA from algae has been reviewed here. EPA is also a kind of ω – 3 PUFA (Polyunsaturated fatty acid); it has a vital part to cure different types of autoimmune disorders, like cardiovascular disease, anti-aging, and for the skin as well. Different research showed uptake of EPA as acrucial dietary supplement to prevent the risk of eicosanoid dysfunctions and uplift the immune system, reduce metabolic disorders, an illness caused by foreign elements and by the body itself [25].

EPA also acts as an anti-cachexia and anti-inflammatory agent.

Application of EPA

The uptake of EPA on a routine base reduces the risk of cardiovascular disease, anti-inflammatory, and anti cachexiogenic benefits [26, 27]. EPA prevents the overexpression of pro-inflammatory cytokines and interleukins, which regulates or plays a key role in immune pathways, but if they overexpress, it causes abnormalities, weight loss, and other disorders. EPA acts as an inhibitor for these cytokines and interleukins, using it protects from the syndrome of cachexia [28, 29]. EPA is a major element for foetal development. Studies show that for fetal brain and eye development during pregnancy, EPA is required [30]. Besides this, EPA also prevents premature birth by interfering in the mechanism of prostaglandin E2 and prostaglandin F2α [31, 32]. It has also been proven that during pregnancy, consumption of EPA reduces allergies, anti-inflammatory disorder and cardiac diseases [33].

Methods of production of EPA

Conventionally, EPA was produced from fish oil, by different methods but because of low yield, peculiar taste, odor, tedious to recover [34]. Hence, nowadays, EPA is produced from microalgae and the biosynthetic pathway is shown in Figure 3. So far for the production of EPA, different species have been used e.g. Phaeodactylum, Nannochloropsis, Thraustochytrium, and Schizochytrium. These species have reported high accumulation of EPA, while Phaeodactylum, Nannochloropsis, reported having 39% of total fatty acids [35]. Table 1 illustrates here the continuous culture, fed-batch, and semi-continuous fermenters are used for EPA production.

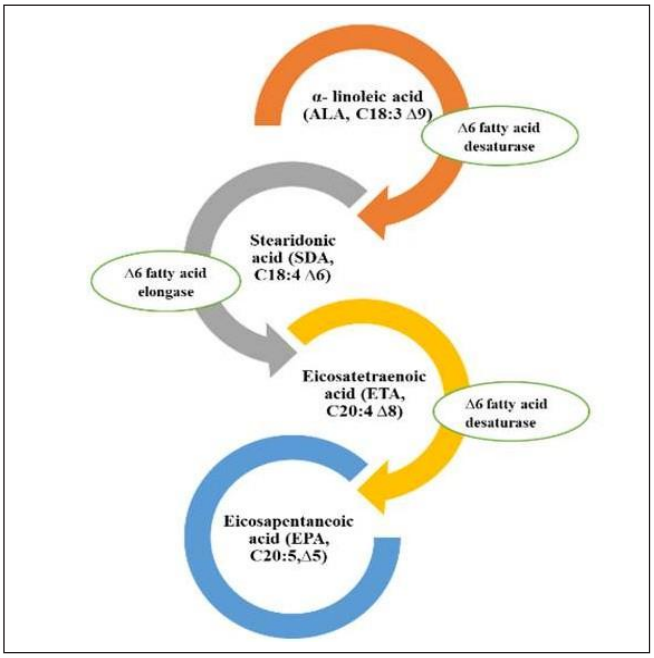

Fig. 3: Biosynthetic Pathway for EPA Synthesis.

Table 1: EPA productivity Comparison in various mode of cultivation.

Culture Process	Organism	Productivity (mg/l/day)	Reference
Batch	*Phaeodactylum tricornutum*	19.0	[36]
Continuous	*Monodus subterraneus*	25.7	[38]
Semi-continuous	*Monodus subterraneus*	58.9	[42]
Perfusion cell bleeding	*Nitzschia laevis*	174.6	[43]
Continuous	*Phaeodactylum tricornutum*	25.1	[37]

However, many strategies had been used to produce EPA. Different fermenter designs and a variety of species have been demonstrated in several studies. Some key investigation for production of EPA is reviewed and many researchers found a method for EPA production from autotrophic algae, while EPA can be produced from heterotrophic algae. P. tricornutumis phototrophic algae when it is used by optimizing conditions like the use of batch culture, modifications in the reactor surface/volume ratio with dilution rate. By adopting this strategy 0.08-0.13 EPA g/l from 3.3 % of biomass was produced [36]. While in some cases, productivity was increased by some parameter in the photobioreactor modifications [39]. It has been demonstrated that the use of heterotrophic algae-like Nitzschia laevi with increasing glucose feed level; productivity measured was 0.073 g/l/d [40]. In the case of Nitzschia alba use of fed-batch along with the use of nitrogen depletion system that increases lipid production which ultimately leads to control in glucose and silicate ratio and also enhances productivity up to 0.3 g/l/ day [41].

Likewise, considering different strategies for other species high yield was achieved for example, in Monodus subterraneus changes in cell density in outdoor culture, productivity enhanced up to 0.059 g/ l/ day [42]. A variety of processes implemented for other species like M. alpine. Some modifications in the process like incubation, adding linseed oil and gain yield 0.1 g/ l/ day [44].

In conclusion, this summary represents different approaches to produce EPA as it demonstrates that phototrophic algae generally possess to high cell density culture. Growth is regulated by light. EPA produced from heterotrophic algae shows higher production.

Production media composition

The composition of the culture medium possesses nitrogen, phosphorus, iron, vitamins, and silicon salts. For P. tricornutum, themain source of EPA production in industries arenitrate and urea used as a goodsource of nitrogen, and yield was reported 2.35 g L−1 day−1 [45], while nitrogen deficiency leads to intracellular carbon flux so that this carbon in no longer capable to further process and formed another product [46]. Iron is essential for photosynthetic activities and performs a role in the electron transport chain. Vitamin B12 required for the growth of diatoms in culture it is a very crucial nutritional growth factor [47].

Downstream processing of EPA

Generally, when EPA produced in crude form, it is amixture of lipids and fatty acids in downstream processing. So, the first step is the extraction of fatty acids from algal biomass. For purification, different phenolic and alcoholic compounds were used in different ratios. There were different methods established for the purification and recovery of eicosapentaenoic acid from microalgae. EPA was purified from the strain of Porphyridium cruentumby saponification of oil, that was done with the mixture of KOH and ethanol incubated at 60°C for 1 hour and then the concentration of PUFA is done by urea method. The methanol and urea were used in 3:1 to crystallize at temperature 28°C. From here concentrated trans-methylated product was formed. Finally, EPA methyl esters were concentrated by a silica gel column chromatography that recovers up to 50.8% EPA separated from PUFA [48].

In the flocculation method, a different type of flocculating agent is used for aggregation of the microalgal cell from suspension. Though the cells are negatively charged, they can be neutralized by using 59. different types of flocculants. Apart from that, flocculant should be non-hazardous, inexpensive, and high yield providing. Generally, multivalent metal ions used, such as alum, aluminum sulfate (Al2 (SO4)3, ferric sulfate (Fe2 (SO4)3, and the ferric chloride (FeCl3). The metal salt efficiency depends on the ionic charge [49]. Despite metal salt cationic polymers (polyelectrolytes) can also be used as flocculant agents. Polyferricsulfate (PFS) is better metal salt as compares to conventional flocculant metal salts [50]. By reducing the charge on the cell surface, they make a bridge by connecting and this process terms as bridging. Polyelectrolyte flocculation activity depends upon the molecular mass, density, ionic strength, and pH. Usually, high molecular weight polyelectrolytes are preferred. Mostly, these polyelectrolyte flocculants are used in the case of chlorella. In some studies, it was found that flocculation can be achieved by changing the pH of broth [51]. Flocculation leads up to 95% recovery of algal biomass from suspension. Thisis not considered a very good method for the purification of EPA, due to loss in the yield [52, 53]. In this method, crystallization of fatty acid occurs due to van der walls forces and break down of the bonds happened [54,55]. In the case of microalgae, the urea crystallization was carried at 28 °C [56]. Filtration works under vacuum or pressure. In this method, different types of filters are used like vacuum filters, membrane filters, and ultrafilters. Here, a precoat layer is formed in which slurry and biomass retain and EPA can be recovered [57,58].

Genetic engineering for EPA production

EPA is an essential omega – 3 fatty acid precursors of another fatty acid, performs a major role in different activities and many more. Due to the lower growth rate, large scale yield is not feasible for industry purposes. Therefore, genetic engineering is required for the commercial production of EPA. Some studies represent the exploitation of marine cyanobacteriafor genetic engineering. Marine cyanobacteria do not produce EPA but by genetic engineering, it can be used as a source of EPA production, [59]. Different strains have been used to produce EPA from genetically engineered species. Shewanella putrefaciensis a marine bacterium that is present in the fish intestine. Shewanella putrefaciensstrain SCRC – 2738 contains gene cluster which is responsible for EPA synthesis is isolated and cloned in the marine cyanobacteriumSynechococcus species that have a higher growth rate. For this cloning, a cosmid vector (pJRD215) is used. Where EPA gene cluster for EPA synthesis is used. This gene of interest is transferred into a cosmid vector then successfully transformation done in Synechococcus which was used as a host. Optimum growth conditions, temperature and culture conditions provided which results in a high yield of EPA [59].Likewiseusing the same strain for EPA synthesis cloning and expression performed in E. coli which also shows good production of EPA even higher than marine cyanobacterium[60].

Research has been undergoing for the synthesis of EPAfrom genetically modified marine bacteria, microalgae are now the main focus is on the bioengineered plant or genetically modified oilseed crops for the synthesis of fatty acids. Some studies represent bioengineered crops that produced EPA. Such as Camelina (Camelina sativa) and Canola (Brassica napus L.) are used as a source for EPA synthesis. Using camelina as a host, it is genetically constructed by using genes from marine microalgae and the authors reported up to 24% EPA synthesis of total fatty acid [61].

Another example of this use is utilizing Canola as a host but by modifying the pathway of EPA synthesis [62]. Usually, EPA synthesis requires desaturase enzyme but here this

method utilizes another pathway. In this pathway polyketide synthase enzyme that can directly biosynthesize PUFA [63]. This pathway is reported in some bacteria and few microalgae. Therefore, by using this method in Canola, EPA synthesis is reported about 0.3 % of the total fatty acid content. These are a few examples of genetically manipulated plants, bacteria, and algae that are using for EPA synthesis and show the successful production of EPA.

Current commercial production of EPA and market potential

\EPA's role in different health activity, as well as a precursor for other fatty acids, makes it very valuable. Therefore, these all health aspects brought this fatty acid in demand in the market. But because of inadequate sources, new alternatives came into existence like microalgae, genetically engineered plants, and bacteria. Research is going on for further improvement and production of EPA. To date, these different strategies have been adopted and new methods are in research. In 2016, the global market of omega – 3 products was reported US$34.7 billion [64]. Currently, EPA is produced from marine fish oil but due to very high consumption, microalgae are also using commercially. Microalgae can produce up to 1.1- 12% dry weight [65].

Carotenoids from microalgae

Carotenoid is a vital pigment responsible for different colors of plant leaves, fruits, and flowers. Besides the higher plants, carotenoid is present in bacteria, algae, yeast, and fungi. Also, Salmon fish and some birds have carotenoid pigments as they obtain it from their food. Carotenoid is a C40lipid-soluble compound. The precursor of carotenoid is an isoprene unit. Carotenoid is a tetraterpenoid compound which has eight isoprene units. Based on the presence of a hydrocarbon and oxygenated group on the structure of carotenoid, it is divided into two groups, carotene, and xanthophyll. Carotene, such as β-carotene consists of a linear hydrocarbon having β-ring at both ends and xanthophyll such as Lutein, violaxanthin, neoxanthin, zeaxanthin is the oxygenated derivatives of carotene [66].

Importance of carotenoids

Carotenoids have an immense role in nature. Apart from pigmentation, it is used in photosynthesis. Carotenoids absorb blue-green light and send it to the reaction center. Carotenoids also cover the apparatus from excess exposure to light [67]. Carotenoid found in foods is very useful for human health. β -carotene is useful for the prevention of cancer and heart disease. It is also important to avoid depression, high blood pressure, asthma, and infertility. β-carotene is used to boost immunity and protect the skin against sunburn. Haemotococcus pluvialis, microalgae produce carotenoid astaxanthin and it has anti-inflammatory, strong anti-oxidant properties.

Application of carotenoids

Due to anti-oxidant property, β-carotene is responsible for chronic Alzheimer's and Parkinson's disease. Chlorella ellipsoidea and Chlorella vulgarisinhibit colon cancer development by the application of their carotenoids [68]. During photosynthesis, reactive oxygen species (ROS) are produced by the mechanism of photoinhibition. ROS contributes to the aging process and plays a part in diabetes, cancer and heart disease. Carotenoids act as a scavenger to the ROS. Due to its antioxidant properties, they are used in the cosmetics industry as well as in the food industry. Apart from the anti-oxidant property, Carotenoids are used widely as a foodcolorant in food and nutraceutical industries. In Australia, Cognis nutrition and health produce 1200 ton/year powder β- carotene used as a nutrient supplement in the food industry, beverage coloring in the beverage industry. The price of the major products β-carotene extracts and Dunaliella powder, derived from Dunaliella salina vary from US$ 300 to US$ 3000/kg. It enhances immunity, anti-aging; prevent cancer, so, β-carotene used as anti-oxidant in the pharmaceutical industry and cosmetics [68]. Also, it has a wide application in agriculture and animal feeding because β-carotene used in feed additive to promote growth and reproduction. β-caroteneis also known as a precursor for vitamin A formation. Thus, it is widely used in varieties of industries.

Application of xanthophylls

Haemotococcus pluvialisis the highest producer of astaxanthin [69]. Astaxanthin has wide applications in nutraceuticals, cosmetics and food industries. For beautifulskin, astaxanthin is widely used in cosmetics, because it restores skin moisture, protects from sunburn, fights against wrinkles and fine lines. Lutein produced from Chlorella vulgarishas an application in the food industry as a food dye and food additive in aquaculture. It has an immense role in poultry farming. Anti-obesity is one of the most important properties of fucoxanthin, this agent burns the fats and reduces body weight. Lutein and Zeaxanthin are rich foods used to reduce the effect of cataract [70].

Method of carotenoid production from algae

The carotenogenic algae are Dunaliella salina, Haematococcus pluvialis,etc. To produce carotenoids, the cultivation of carotenogenic microalgae is needed ona large scale. The main purpose of cultivation is the high yield of biomass with the content of carotenoid. Different types of methods are applied to produce cost-effective carotenogenic microalgae in a higher amount. Generally, two methods are designed – Open Method and closed method [71]. Under the open system, Cultivation is done in ponds and other varieties. The alternative way of the open pond in the open trench, in which flow keeps the culture stirred and provides continuous harvesting. Under the closed method, biomass production in a photobioreactor (PBR) is designed. As the open system is

contamination susceptible, the cultivation of Dunaleila salina is possible because it is contamination resistant halophilic microalgae; whereas Haematococcus pluvialis, is cultivated in photobioreactor due to its sensitivity towards contamination. Photobioreactor though is not economically viable; it provides a control condition for the cultivation of carotenoid microalgae [72]. The two steps process is employed in the accumulation of carotenoid. The first stage is the batch cultivation. Carotenoid producing cells are grown under optimal conditions. After the growth level is reached at the saturation point, the biomass is subjected to stressful conditions [73]. The accumulation of primary carotenoids depends on photosynthesis; on the other hand, secondary carotenoids such as astaxanthin are achieved under a stressful condition such as the addition of ROS, mineral starvation [74, 75]. The secondary carotenoids are biosynthesized in Haematococcus pluvialis by triacylglycerols accumulation Figure 4. The precursor of carotenoid synthesis is isopentenyl pyrophosphate [76].

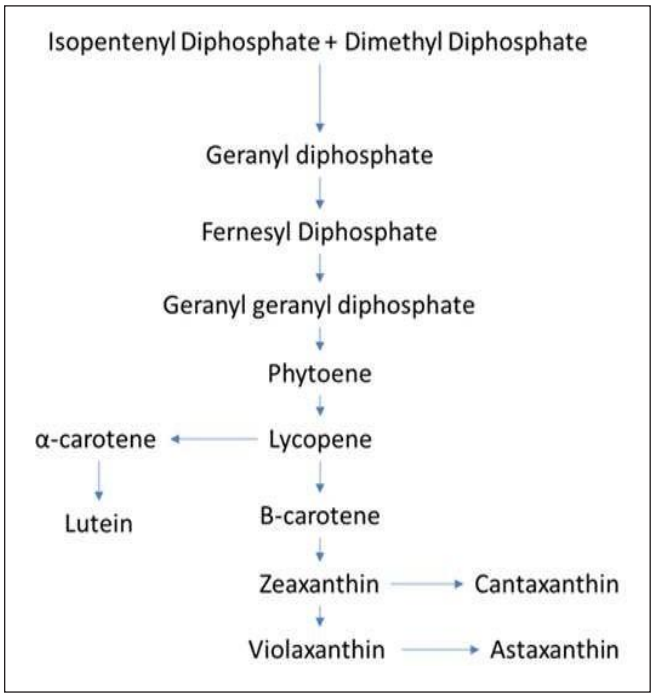

Fig. 4: Biosynthesis pathway of carotenoids in Algae.

Media composition for carotenoid production

Freshwater culture media used for production is categorized into three types: synthetic, enriched and soil water media. Synthetic is designated as BG-11 and WC. Synthetic enriched media is enriched by adding the soil/plant extract. Some enriched media are Alga-gro lake, Auduinella, Diatom medium, VS and soil 61. water media are composed of 1 or 2-part algal grown soil added with 3-4 part of deionized water. Sometimes, some metals such as calcium carbonate (for phototrophic freshwater algae), Ammonium, Magnesium, Phosphate hexahydrate (Botryococcus and euglenoids) has been added [77]. Media composition for freshwater culture such as Fraquil media is supplemented with EDTA, Zn, Fe, Mn, Co, Cu, Na2MoO4.2H2O, vitamin, biotin, and thiamine. Similarly, sometimes called marine culture media is prepared using seawaterdirectly.

Downstream processing of carotenoids

Downstream processing is mainly worked to extract the product from biomass. This is done through harvesting, extraction, purification, quantitative and qualitative analysis. Harvesting of biomass can be done by sedimentation, filtration, flocculation, or centrifugation. For Open pond cultivation, flocculation and surface adsorption are used for harvesting. Cells form clumps and easily settle down during the process flocculation. For appropriate flocculation, flocculation reagent is needed such as FeCl3 or Al2 (SO4). Some microalgae such as Chlorella minutissma can produce flocculation without adding reagent, the result of which is much easier to separate by filtration or low-speed centrifugation. For large cells such as H. pluvialis, the combined process of sedimentation and centrifugation is required. For photobioreactor, centrifugation is done for harvesting [78]. Sedimentation can also be done under the action of gravity or low-speed centrifugation. For the separation of large cells, the diatomite and cellulose filters are used and for the smaller cells such as Chlorella needs membrane filters. Filtration depends on the size of the organisms and the viscosity of the medium.

Cell wall disruption

The first step of the downstream process is the cell wall disruption; it is followed by extraction and purification [79]. Cell wall disruption is done through physical, chemical and enzymatic methods. Physical cell wall disruption methods are done through grinding, heat shock, high-pressure homogenization; chemical cell wall disruption is attained through alkaline, an organic solvent or by detergent. In enzymatic cell wall disruption, an enzyme such as lysozyme disrupts the cell wall [80].

Biomass Extraction

Carotenoid is obtained through the extraction of microbial biomass. The conventional technique used in the extraction of carotenoids is extraction with organic solvent [81]. The organic mediated solvent is used for pigment extraction. Non –polar solvent hexane is one of the organic solvent used. Dodecane and methanol are used to extract Astaxanthin from H. pluvialisis. For efficient extraction, a supercritical CO2 method is used. Supercritical fluids exist above the critical temperature and pressure [19, 66]. The limit of this method is the contamination with chlorophyll and the requirement of expensive equipment. Supercritical fluid extraction 62. with CO2 permits extracting a large variety of lipophilic compounds such as Carotenoids. Supercritical CO2 at 20 MPa and temperature 40°C extracts approximately 80%

carotenoid content. Supercritical CO_2 extracts canthaxanthin and astaxanthin from Chlorella vulgaris and high yield of β-carotene from Dunaliella salina. Supercritical CO_2 with a co-solvent (ethanol) enhances the solubility of compounds and reduced the interaction with the matrix [82]. Solvent-based extraction of lipids is shown in Table 2.

Table 2: Biomass extraction through various methods.

Extraction	Solvent Used	Yield of Extraction	Carotenoid	Source of Extraction
Organic solvent-mediated extraction	Hexane	95%	Lutein	*Scenedesmus almeriensis*
	Dodecane and Methanol	85%	Astaxanthin	*Haematococcus sp.*
Green–solvent-mediated extraction	Olive oil	93.9%	Astaxanthin	*Haematococcus sp.*
Supercritical Fluid Extraction	Carbon dioxide	25%	Astaxanthin	*Haematococcus sp.*
	Carbon dioxide + ethanol	7.62 mg/g	Total carotenoid content	*Nannochloropsis occulata*
	Carbon dioxide	---	Cathaxanthin, Astaxanthin	*Chlorella vulgaris*

Genetic engineering for carotenoid production

Genetic engineering of microalgae is the advance technique to modify the biosynthetic pathway for increasing the yield. The four major transformation techniques are bombardment, electroporation, glass beads and Agrobacterium transformation. Nowadays, CRISPR Cas used as an editing tool for genetic engineering. Three components must be standardized for the genetic transformation into algal cells: promoter, selectable marker and expression vector. Phytoene desaturase gene (PDS) determines the carotenoid production. The enzyme phytoene synthase (PSY) catalyzes the geranyl-diphosphate to produce 15-cis-phytoene. The isolated PDS gene transferred to the E. coli to express and PDS was able to convert phytoene to β-carotene. PDS is up-regulated by high light and glucose. PDS mutation is also done for changing of an amino acid to become it as herbicide norflurazon resistant, [83].

Mark Harker worked on the keto-carotenoids biosynthesis in transgenic cyanobacteria. Haematococcus pluvialis produces canthaxanthin. In H. pluvialis, the gene crtO encoding for β-C-4-oxygenase is used to convert the β-carotene to canthaxanthin. This gene crtO was inserted into Synechococcus PCC7942. The transgenic Synechococcus with crtO produced astaxanthin as well as zeaxanthin [84, 85].

Current commercial production of carotenoids and market potential

Carotenoid has wide application and thus has market potentials. Carotenoids are mostly used in the segment like feed, food supplement, cosmetics, and pharmaceuticals. Based on the type, carotenoid markets are segmented into astaxanthin, β-carotene, canthaxanthin, lutein, lycopene, and zeaxanthin. Till 2016, the market value of carotenoid is $1.24 billion and expected to $1.53 billion in 2021. The highly demanded carotenoid is astaxanthin produced by the culture of H. pluvialis. However, synthetic astaxanthin dominates the global market exceeding value $200 million with 130 metric tons per year. Astaxanthin producing companies are Cyanotech corporation, Mera pharmaceuticals lnc.,ValensaInternation, Fuji chemical industry co. ltd. etc. In India, Parry nutraceuticals brand named Zanthin produces Astaxanthin. The estimated market value is $2500-7000/kg to $15000/kg astaxanthin from H. pluvialis whereas production cost is $1000/kg. Due to a large number of end-use applications of carotenoids, the market value of it rises gradually. Major types of carotenoids called β-carotene, zeaxanthin, lutein, and astaxanthin are high in demand products worldwide. In 2013, the global market value of β-carotene reached $280 million which is estimated to grow by 3.5% from 2014 to 2019 [86]. As the consumer becomes more aware of anti-aging, anti-cancer, and other health issues, carotenoids will take a major position in the global market.

Production systems for algal products

There are various kinds of photobioreactors that have been designed accounting for the substrate utilization, scale-up and feasibility of resources. Photobioreactors are categorized as

flat-panel photo-bioreactor, dome-shaped, flat-panel airlift, bubble column, airlift column, helical type, stirred tank and tubular type photobioreactors [87, 88]. Tubular bioreactors are mostly designed for the large-scale cultivation of micro-algae with controlled conditions in an open area. For designing any kind of photo-bioreactor, the physiochemical and biological parameters must be concerned such as light requirement, volumetric oxygen flow rate, surface area, carbon dioxide consumption, substrate concentration, pH, temperature, size of inoculums, growth stability, etc [89]. Figure 5 describes the schematic representation of different kinds of cultivation systems.

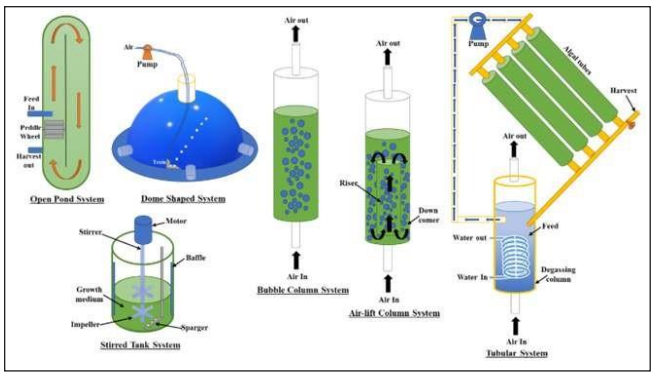

Fig. 5: Different kinds of production systems for algal products formation.

The Chlorella sp. was used by Rasoul et al. in a 2L bubble column bioreactor to produce different fatty acids and esters [90]. Haematococcus pluvialis was used to produce astaxanthin with the optimization of a specific light uptake rate (qe) of 4.5 X 108 µE per cell per second [91, 92]. Chaetoceros calcitranswas used by Krichnavaruk et al. in a 17-liter airlift photobioreactor for them with an 8.88 x 106 cells per ml [93]. Bubble column reactor was used for the astaxanthin production by Haematococcus pluvialis.The air-lift bioreactor was used with a production reached to 600 mg/l using 7x106 cells /ml [94]. Phaeodactylum tricornutum UTEX 640 was grown toproduce EPA by Grima et al. under a batch process. The maximum biomass achieved was 25 g/l [95]. In a continuous mode of cultivation of Phaeodactylum tricornutum in air-lift driven tubular reactor, the productivity was observed at 1.2 g/l/d [96].

Conclusion

The high-value product formation by the use of microalgae is a low-cost biological process, in which micro-algae is required to be grown in photobioreactors. Various parameters of micro-algal cultivation such as growth kinetics, different kinds of photobioreactors, designing and maintaining their operational parameters are required to be optimized. It is concluded that the study about the biosynthetic pathways, production medium composition, andfeasible extraction methods are the additional credits for optimizing the process of development of high-value products like DHA, EPA, and carotenoids from micro-algae.

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SAVE THE ENVIRONMENT (STE) was founded and registered on 19th November 1990. In 1992 with the collaboration of WWF (India), the organization started working to combat arsenic poisoning problem of water in the arsenic prone areas of West Bengal. Since then STE has been involved in various projects related to combat arsenic problem in India.

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To protect present and future generations from various environmental hazards.

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To create awareness and motivation among rural communities & provide cost effective, energy efficient & environment friendly technologies.

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