CHAPTER 2

LITERATURE REVIEW

2.1 Hot Springs

Hot springs or thermal springs are springs with water at temperatures considerably higher than the surrounding air temperature. Most hot springs discharge groundwater that has been heated by shallow interruptions of magma in volcanic areas. However, some thermal springs are not correlated to volcanic activity. The color of the water in hot springs is a result of thermophilic microorganisms made up of cyanobacteria, archaea, and algae. Various thermophilic organisms grow in huge colonies called mats that form the colorful scum and slime that appear on the edges of hot springs. Along with geochemistry, the temperature and pH values of hot springs play an essential role in defining which organisms live in them. Hot spring thermophilic algae are most abundant at temperatures of 55°C or below. The potential energy sources used to determine algae growth are various and include carbon dioxide, water and daylight.

Many hot springs occurred in 29 provinces of Thailand and most of them are located in northern Thailand such as Chiang Rai, Chiang Mai, Mae Hong Son, Lampang, Lamphun, Phrae, Nan, and Sukhothai province (Figure 2.1). The Department of Mineral Resources (1987) reported that rock formations and crevices are the cause of some of the geothermal resources in the north of this country. These cause heat to spread between the slides and splits of stones and bedrock. At that point, the hot magma underneath the earth’s surface transfers the heat to the cold water, which permeates into the surface of the earth and changes the cold water into boiling water which then afterwards rises up through the crevices and crevasses of the hot magma. Then, the water stream will spring up through the ground (Figure 2.2). Hot springs create steam or discharge in various ways which are unique to each zone. The temperature of the hot spring water that rises to the surface is typically between 45-100°C (Castenholz 1973).

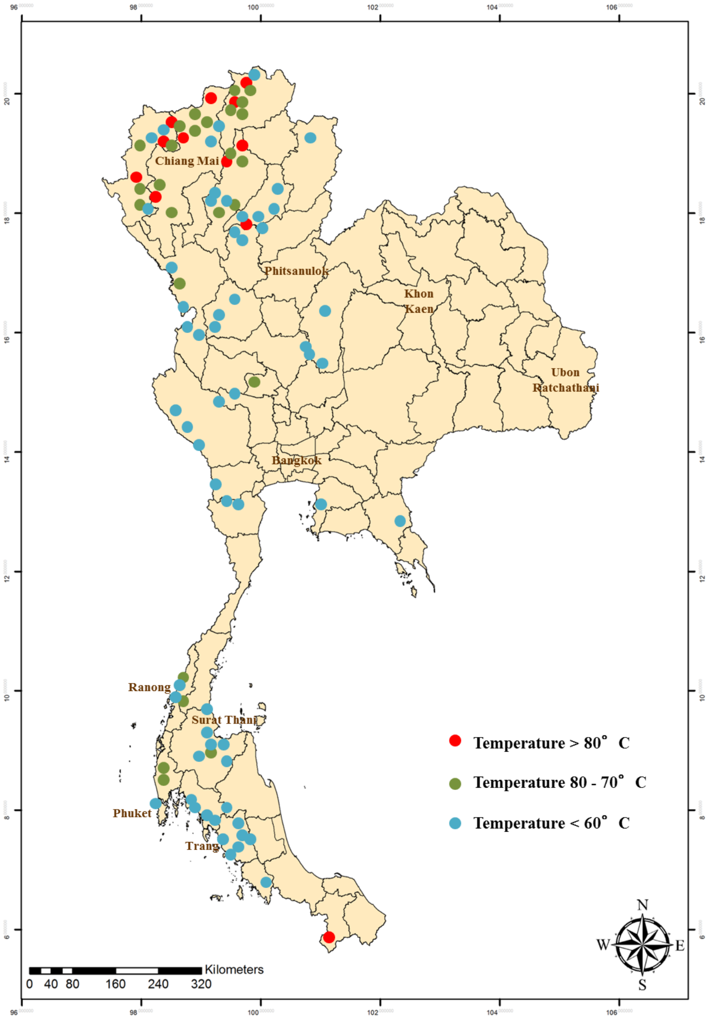

Figure 2.1 The distribution of hot springs in Thailand classified by temperature range (By Geological Society of Thailand)

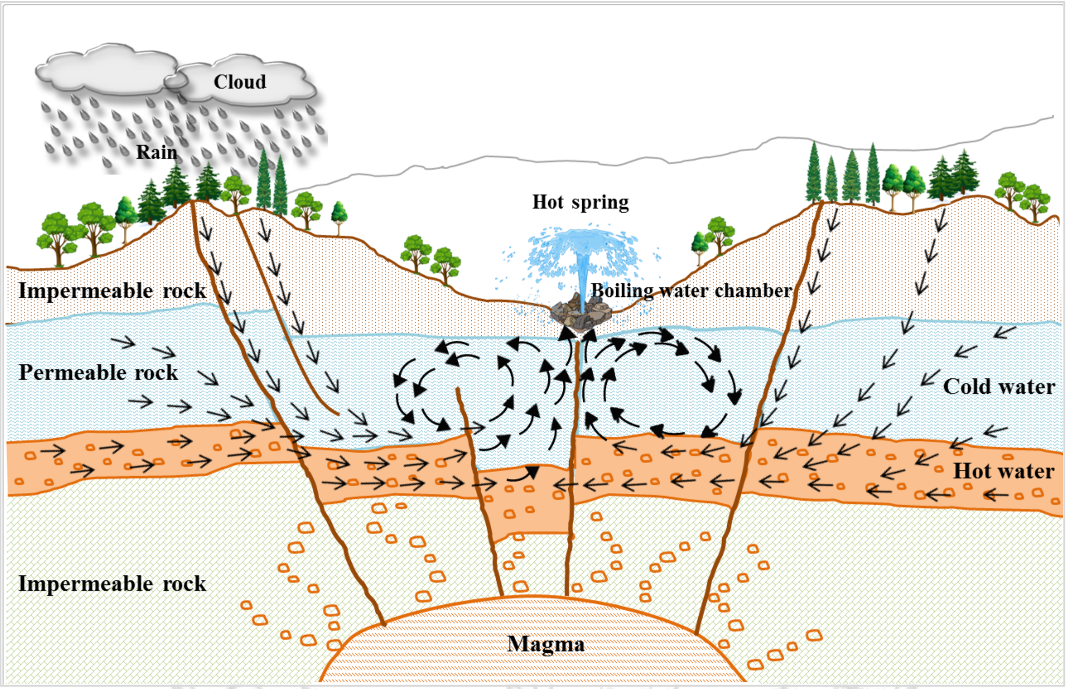

Figure 2.2 The natural cycle of hot springs and geothermal vitality in northern Thailand modified from International Network for Sustainable Energy (INFORSE)

2.1.1 Hot springs can be classified according to a variety of categories including temperature, pH, usage and the process of occurrance (Subtavewung *et al.* 2005).

2.1.1.1 Temperature classification

The classification of hot springs by temperature is presented in Table 2.1. From the one hundred and nine hot springs found in Thailand, 41 hot springs (37%) are reported to be thermal springs, whereas 68 hot springs (63%) have been identified as hyperthermal springs.

Table 2.1 Types of hot springs classified by surface temperature

Type	Temperature (°C)
Cold spring	T < 20°C
Hypothermal spring	20°C ≤ T < 30°C
Thermal spring	30°C ≤ T < 50°C
Hyperthermal spring	T ≥ 50°C

2.1.1.2 pH classification

Hot spring waters can be classified by pH as being acidic, basic, or neutral, and also from the balance of hydrogen that is present in the water (Table 2.2). However, the hot springs found in Thailand are limited to 3 classes including neutral, weak alkaline and alkaline. Twenty-four percent of Thailand's hot springs show a neutral pH value (pH = 6-7.5). Weak alkaline hot springs, which are in a pH range of between 7.5 and 9 make up 68% of the hot springs in the country, and the last 8% are classified as alkaline hot springs (pH = 9-10).

Table 2.2 Classification based on pH as divided into 6 classes

Type	pH
Strong acid spring	pH < 2
Acid spring	2 ≤ pH < 4
Weak acid spring	4 ≤ pH < 6
Neutral spring	6 ≤ pH < 7.5
Weak alkaline spring	7.5 ≤ pH < 9
Alkaline spring	pH ≥ 9

2.1.1.3 Usage classification

It is important to know the status of all hot springs, if the sustainable development of their relevant geothermal properties is being investigated. Hot springs could be classified by usage into 4 types including;

Table 2.3 Classification based on usage as divided into 4 classes

Usage	Court
Power plant	1
Tourism	49
Consumption	19
Unutilized	45

2.1.1.4 Process appearance classification

Geysers are hot springs that discontinuously and savagely discharge a jet of boiling water and steam from a hole in the ground. Many geysers launch steam and high-temperature water several meters into the air; however, there are a few that can send jets of water of up to 60 m over the ground. A number of geysers discharge water and steam at normal intervals, while others are extremely changeable. Geysers are divided into two groups: those where water is ejected from a cone-like hill, and those where wellsprings of water are launched out in various headings from a canal to form a pool.

Hot springs are pools of warm or heated water that are issued from springs. In this case, the spring is unrestrained so that the stream is nonstop. Surface water returns to beneath the ground to maintain the circulatory flow. Pools are regularly bordered with a hard siliceous or calcareous deposit that has been precipitated from mineral springs. Springs that are located near volcanic holes are routinely extremely acidic. On the off chance that the acidic spring water has gone through limestone, which will collapse as a consequence, redeposition of the calcite results in the formation of limestone that has been deposited by the mineral springs. The warm and hot pools can bolster thermophilic living beings on a miniaturized scale. These life forms frequently give the edges of the pools a beautiful yellow, blue-green, orange or dark-colored coloration. Hot springs occur normally in dynamic volcanic ranges, but likewise happen in non-volcanic locales where permeating water comes down into areas where the stone is hotter as a result of the geothermal gradient.

Fumaroles, which refer to fumus, the Latin word for smoke, is a stream of vapor, which changes to steam with minor volcanic gasses. It irregularly and brutally is ejected from a gap in the ground. Fumaroles happen where underground water supplies are constrained. Fumaroles frequently happen on the sides of volcanoes, at higher heights than geysers and hot springs.

Mud pots are hot, sloppy pools of gurgling wet clay. Like fumaroles, these happen where the water supply is restricted. Purple sulfur microscopic organisms utilize the hydrogen sulfide gas and change it to sulfuric acid, which disintegrates shake minerals and create clay.

Seite (2013) has described the thermal waters as cosmeceutical, such as in the case of La Roche-Posay thermal spring in France. This study found that the thermal waters displayed antioxidant, immunomodulating and anticarcinogenic effects, while also having anti-inflammatory and anti-irritant potential. She justifies the use of selenium-rich thermal spring water as an active "cosmeceutical" ingredient in topical formulations. Sherpa *et al.* (2013) presented a complete detailed study of the chemical and physical properties of hot springs while examining the three hot springs of Sikkim. Water from the Tatopani has been claimed to be able to cure many diseases. Hence, the chemical composition of this thermal water should be recognized for its beneficial use. The findings show that the seasonal variations in the physicochemical parameters of Polok, Borong and Reshi Tatopani should be studied in the future to understand the changes in the chemical constituents of the water samples. Any study of hot springs should involve the crucial aspect of relating their balneotherapeutic properties with the enhancement of the Medical Tourism of the state. It has been estimated that thirty different physicochemical parameters of the water samples collected from these study areas were analyzed.

2.2 Factors influencing the distribution of microalgae

Living organisms thrive in appropriate environmental conditions (Primack 2000). Several environmental factors influence whether algae can grow. There is not only one property that limits algae growth, but growth, in fact, depends on the dynamics of an ecosystem (Goldman and Horne 1983). The ecological dynamics involve the relationship between three main factors, which are the physical, chemical and biological factors that are present in each ecosystem (Kochasany 1993). In extreme ecosystems, not only do algae live in the benthic form (Hynes 1970), but phytoplankton communities have been found to exist as well. The microorganisms are able to grow on any surface of substratum. However, the type of substratum, whether it may be rock, sand or silt mud, affects their behavior. The substratum type is related to both the current and volume of water (Peerapornpisal *et al.* 2001). Therefore, the characteristics of the substrata have directly impacted the distribution of microalgae (Chapman 1973). The distribution of benthic algae is usually found in high abundance among rocky and cobble substrates.

Benthic diatom distribution can be presented as an indicator of the ecosystem of the water body. A key issue for understanding diatom distribution is controlled by the geographical factors that limit species dispersal, as diatoms are restricted only by the capability of the species to grow under a specific combination of environmental factors (Kristiansen 1996). The factors include physical barriers, the distance between suitable habitats, the transportability of cells, the type and importance of the transportation mechanism, as well as the size and distribution of the original populations that are suitable for colonization. Species spatial distributions, as well as their abundance, are often recognized for the breadth and position of their niches. Species are typically present in places where their requirements are fulfilled. On the other hand, a species may be present even at a site where its niche supplies are not met and population growth is negative. The population may be maintained by a continuous supply of individuals from neighboring sites with positive population growth. Therefore, species spatial distribution is determined by species niches and available habitat distributions, as well as by spatial population dynamics and dispersal limitations. Jonker *et al.* (2013a,b) estimated the algal diversity in thermal springs in Limpopo Province to contribute to the broader understanding of the distribution, occurrence and exterior factors that influence specific genera in the hot spring waters of South Africa. The researchers assessed six sites of thermal water, which were analyzed for their physical and geological properties that may affect the species diversity within these thermal waters.

2.3 Diatoms

Diatoms are a type of benthic algae in the Division Bacillariophyta. The sculptural parts of the cell walls are composed of silica (Foged 1977). Diatoms have a special characteristic of cell structure known as frustules. The frustules include bi-valves. The large valves are called epitheca, and the small valves are called hypotheca. Bivalves are composed of pectin and silica. The exterior color of the diatom is yellow-brown as a result of the color of the chromoplasts found inside. A single diatom cell is approximately 5-500 µm in diameter. The shape of the diatom can be classified as either symmetrical or asymmetrical. The valve structure is called striae and includes a range of small pores of punctae and longitudinal valves called raphe (Figure 2.3). These characteristics are a major feature used in their identification.

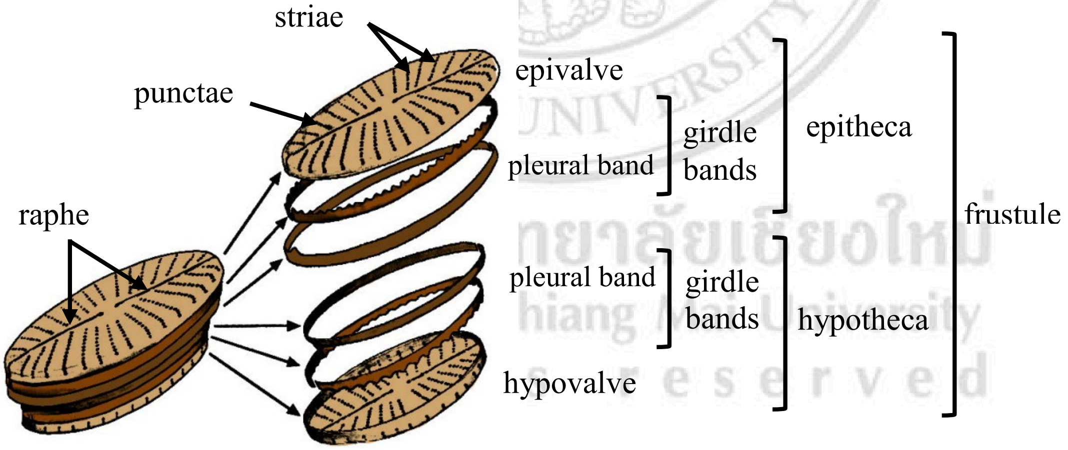

Figure 2.3 Diatom cell structure modified from Ian Nettleton

Diatoms can be found in both freshwater and saline water in the form of periphyton and plankton. They live as a brown-colored biofilm on the substrate, while some of them live as a filamentous colony (Chapman 1973; Barber and Haworth 1981; Kelly and Haworth 2002). Diatoms are considered potential bioindicators of water chemistry because of their ecology, and as a consequence of their differing levels of tolerance to the variable factors that can limit diatom distribution. This may result in their eventual succession. In comparison with benthic macroinvertebrates, diatoms are considered more sensitive indicators of water chemistry due to their shorter life cycles and their nature as primary producers (Steinberg and Schiefele 1988). Moreover, diatoms are common in the attached algal communities of streams.

2.4 Diatom Identification

In 1974, the systems and principals of diatom diagnosis or identification were established. The system is a standard diagnosis of diatom taxa, which has been designed as an organized outline for any author who has to describe diatoms. All diagnoses should comply with the International Code of Botanical Nomenclature (ICBN). So, the frustule of diatoms should be diagnosed by considering the major structure along with certain other information. Examples of this would include valve outline, length, width, protoplast, habitat information, areolation, striation, relative size, apical and marginal fields, processes, reproduction and ecological distribution (Anonymous 1975; Ross *et al.* 1979). In the present time, cell structure, life cycle, and habitat information are considered the essential features of diatom study. The important findings of diatom studies could be effectively applied worldwide, such as by using diatomites as filters in industrial processes, like in pesticides in agricultural applications, as drug carriers in the medical field and in environmental monitoring.

2.5 Thermotolerant diatoms

Thermophilic and thermotolerant microorganisms, which could grow at high temperatures, have been found in and around hot springs. Attached algal communities consist primarily of microscopic species, which are particularly found in small streams and on rocky substrata. These include several genera of Cyanophyta and Bacillariophyta (Smith and Smith 2001) such as diatoms. Diatoms are considered very suitable bioindicators due to their tolerance to the variable factors that limit their distribution, and this may result in their eventual succession (Round *et al.* 1990). Morales *et al.* (2011) made comparative study results of freshwater and brackish diatoms collected from hot springs in eastern Russia, notably at several locations of the Kuril Islands and on Sakhalin Island, and compared the results with others investigations on diatoms collected from hot springs on Shiashkotan, Yankicha, and Sakhalin Islands. They reported that the study of the Kuril Islands hot springs, and the water courses formed by them, revealed the presence of diatoms in 145 species of 3 classes, Coscinodiscophyceae, Fragilariophyceae, and Bacillariophyceae. The study on the Sakhalin Island hot springs revealed the presence of diatom flora in 125 species of 3 classes, respectively. In the represented genera, *Pinnularia* with 22 taxa, *Nitzschia* with 20 taxa and *Navicula* with 23 taxa were recorded.

Diatom species allow for a wide range of temperature for growth from 10°C to 45°C in extreme habitats according to Round *et al.* (1990) who found that this was higher when compared to other green algae (Adenan *et al.* 2013). Moreover, the distribution of diatom species is allocated within the range of the low and high limits of the conductivity tolerances (Hamed 2008). Thus, the selection of suitable microalgal species for cultivation purposes while conserving the growth rates could support the eco-design of an efficient and sustainable production chain (Lardon *et al.* 2009).

Benthic diatoms that are found in extreme habitats such as hot springs have been studied in a number of research studies. The diatom floras obtained from geothermal environments in the hot spring systems of Iceland, New Zealand, and Kenya were examined. Owen *et al.* (2008) reported that a significant factor for geothermal floras include temperatures of lower than 45°C. At that condition, 251 species were observed, of which *Pinnularia* sp., *Navicula* sp., *Anomoeneis* sp. and *Fragilaria* sp. were the most common. Statistical analyses have suggested that the diatoms cluster into seven major groups and Canonical Correspondence Analysis (CCA) indicated less strong correlations for silica and temperature with regard to species, alkalinity, pH, and conductivity values, whereas the stronger correlation factors included substrate type, current velocity, and light conditions. Cocquyt and Van De Vijver (2007) studied the diatom composition of a Peruvian hot spring in the Colca Canyon, where *Synedra pseudogoulardii* Manguin was the most abundant species and its diversity increased with decreasing temperatures.

Additionally, Rousch *et al.* (2003) studied the changes in the fatty acid composition of marine diatoms, and studied comparisons between thermo-intolerant (*Phaeodactylum tricornutum*) and thermo-tolerant (*Chaetoceros muelleri*) species during conditions of temperature stress. The results found that heat showed a greater effect on *C. muelleri* than on *P. tricornutum*, in terms of fatty acid composition and the degree of fatty acid saturation. Furthermore, the temperature conditions in particular had a more significant effect on changes in fatty acid composition. Variations in fatty acid composition and the degree of fatty acid saturation occurred more quickly in diatoms in response to increasing temperatures than previously observed under conditions of nutrient starvation.

2.6 Benefits of thermotolerant diatoms

Parkinson and Gordon (1999) revealed that diatoms have significant potential in the production of a wide range of materials that may be of great benefit to the fields of nanotechnology and microfabrication. The diatom can be cultured in conditions leading to the creation of random mutations such as by adding a chemical mutagen or subjecting the living diatom culture to UV light. Diatoms attaining the desired characteristics could then be cloned. There are several advantages of diatom frustules for instant gel filtration, biosensors, immune-isolation or microfabrication. Diatoms have been used in toxicity testing (Atazadeh *et al.* 2009). Besides, they have also been used in biomineralization, the synthesis of biomaterials and in the degradation of waste that can provide useful indications in terms of environmental risk assessments of toxicity test compounds (Berard *et al.* 2002). Additionally, Aw *et al.* (2011) investigated silica microcapsules obtained from diatoms as a new carrier for the delivery of therapeutics. In this study, two drug models were used and investigated involving a hydrophobic (indomethacin) and a hydrophilic (gentamicin) model. The results showed the effectiveness of diatom microcapsules for drug delivery applications, showing sustained drug release over 2 weeks and a 14-22 wt% drug loading capacity. These results indicate that drug-delivery applications can be successfully applied to natural materials based on the presence of diatom silica, and this finding offers considerable potential for these materils to replace existing synthetic nanomaterials. In this application, diatom valves can be well preserved and provide a record of past and present environmental conditions, as they can be used in a range of applications in forensic geoscience (Cameron 2004). The identification of diatoms is in the body tissue of corpses can prove whether or not the death occurred from drowning (Bortolotti *et al.* 2004). Furthermore, the axenic culture of diatoms has an advantage in biotechnological applications as polyunsaturated fatty acid producers (Wen and Chen 2002) and as producers of other biological molecules (Lebeau and Robert 2003). These advantages involve the specific structure of diatom-bacteria interactions and the characteristics of the extracellular polysaccharide matrix of diatoms (Chiovitti *et al.* 2003).

2.7 Diatom cultivation

Many techniques have been developed for diatom isolation and cultivation. Kimura and Tomaru (2013) tested the simple decantation method for dispersing cells on flat agar plates for comparison of the finely dimpled surface by printing on the agar plate with nylon mesh patterns for the culturing of *Cheatoceros tenuissimus* Meunier. Cells were grown in modified SWM3 and cultured under a 12/12-h light-dark cycle with cool white fluorescent illumination at 15°C. The results showed that *C. tenuissimus* are easily retained in the dimples, resulting in uniform cell growth over the plate surface. In contrast, on the smooth surfaced plate, the cells survived at only a fraction of the survival rate and made small colonies during the nine days of culturing. Safonova *et al.* (2007) said that the cultivation of diatoms on a micro-scale, and the automatic cell counting process by digital micro-photographs based on ImagePro software, proved to be a convenient technique in the study of the effects of mercaptoethanol and Cu^{2+} on *Asterionella formosa*, and Cu^{2+}, Hg^{2+}, Cd^{2+} and phenanthroline upon the growth of *Synedra acus*. Zheng *et al.* (2005) studied the effects of potassium iodide (KI) on the growth and metabolite accumulation of *Nitzschia closterium* (Ehr.) W. Smith and *Phaedactylum tricornutum* Bolin to evaluate the potential applications to the mass culture of the two diatoms in open environments. Supplementation of 1000 $mg \cdot L^{-1}$ concentration KI resulted in a reduction of the induction phase and led to an increase in the accumulation of biomass and extracellular polymeric substances.

2.8 Effect of physical factors on diatoms

2.8.1 Light intensity

In an ecosystem, the light intensity is a limiting factor in a different group of algae (Hynes 1970). Seasonal changes in light, together with accompanying changes in temperature and precipitation, bring about seasonal changes in any recurring biological events. Factors that affect the cell density include liquid circulation time, hydrodynamic stress, light regime, and mass transfer (Barbosa *et al.* 2003). Light regions are closer to the sun's light and result in the algae shading one and other creating dark regions. For diatom communities, light is one of the factors that would limit the growth rate of the diatoms. Since diatoms live in intimate contact with substrates, the organic carbon in the sediment may be of nutritive value for the diatoms. The growth rate of diatoms in the culture is strongly reduced by the short lengths of exposure to daylight. Furthermore, the low daily irradiances reaching the sediment surface limited the autotrophic growth rate of the diatoms on the intertidal flats.

In terms of the growth factors, light in terms of quantity and quality is the main growth-limiting factor of photoautotrophic microalgae (Vadiveloo *et al.* 2017) and is the source of energy that efforts the photosynthesized reaction. In this effect, intensity, photoperiod and spectral quality requirements need to be considered. For instance, microalgae assimilate inorganic carbon for conversion into organic matter. Light intensity plays an essential role, but the requirements vary widely in terms of the density and culture depth of the algal cultivation. At greater cell concentrations and higher depths, the light intensity must be amplified to infiltrate through the culture. For instance, 18.5 μmol m^{-2} s^{-1} is appropriate when using Erlenmeyer flasks. Light may be natural or supplied by fluorescent tubes. The photo-inhibition may be the result of too high a degree of light intensity (*e.g.,* direct sunlight with a small container close to artificial light). Likewise, overheating caused by both artificial illumination and natural sources should be evaded. The most active portions of the light spectrum for photosynthesis is fluorescent tubes that emit either in the blue or the red light spectrums (Lavens and Sorgeloos 1996). Su *et al.* (2017) studied the effects on the frustule morphology of the diatom *Coscinodiscus granii* at different light spectra. After 10 months of cultivation, the results revealed that the size of *C. granii* frustules and the foramen size increased for all light treatments. In a comparison of the levels of light intensity, the main difference was seen in the foramen density in the short-term treatment, while the lowest density was established under the high-intensity red light. Thus, the mean foramen density and foramen diameter were statistically similar for the short- and long-term tests under red lighting at 300 μmol photons m^{-2} s^{-1} and white lighting at 100 μmol photons m^{-2} s^{-1}. The foramen density was significantly reduced at 100 μmol photons m^{-2} s^{-1} for all color wavelengths. The foramen density was also reduced under blue and white light at 300 μmol photons m^{-2} s^{-1} after long-term cultivation periods as compared to periods of short-term cultivation. Sicko-Goad and Andresen (1991) studied the effects of growth and light/dark cycles on the diatoms *Cyclotella meneghiniana*, *Meloseira varians* and *Stephanodiscus binderanus,* as well as lipid content and composition under the three light regimes (16:8h, 20:4h and 12:12h) at 20°C. *M. varians* and *S. binderanus* demonstrated a short-day preference (12:12h) for growth, whereas *C. meneghiniana* displayed a long-day preference (20:4h). The lipid composition of the diatoms was related throughout the growth cycle. The aged (two-month-old) cell was high in total lipids and triacylglycerides. The interplay between light and temperature as they affect algal growth was reviewed by Round (1973) who claimed that some algal species exhibit day-length preferences. Round went on to say that day-length optima might be related to temperature, while some widely occurring species may not demonstrate day-length optima.

For lipid production, Opute (1974) studied two freshwater diatoms, *Nitzschia palea* Kütz, *Navicula muralis* Lewin, and one marine species, *Navicula incerta* Grun, for their lipid and fatty acid contents. Diatom lipids, whether grown in the light or the dark, were identical in terms of quantitative differences. More storage lipids such as triglycerides were produced in the light than in the dark. Shi *et al.* (2016) studied the growth and physiological characteristics of eight strains of diatoms isolated from aquatic habitats with contrary water column characteristics. The experiment was examined under variable light intensities to compare variations in degrees of low and high light intensity. Diatoms were isolated from low-light-adapted (LLA) and high-light-adapted (HLA) conditions based on their photoacclimation characteristics and differences in growth. The higher growth rates and higher levels of photosynthetic activity were found in LLA diatoms at minor light intensity levels (2, 12.5, and 25 μmol photons$\cdot$m$^{-2}\cdot$s^{-1}). HLA diatoms displayed higher growth rates and higher photosynthetic levels of activity at higher light intensities (60 and 80 μmol photons$\cdot$m$^{-2}\cdot$s^{-1}). The adaptation of habitat-specific differences to light among diatoms initiated alterations in light intensity for the diatom formation in both low-light ecosystems and high-light ecosystems. Grouneva *et al.* (2016) inspected how irradiance fluctuations influence the relative abundance of the main photosynthetic proteins in the centric diatom *Thalassiosira pseudonana* using mass-spectrometry-based methods for relative protein quantitation. The fluctuating light conditions resulted in a generous overall up-regulation of light-harvesting complex proteins along with some subunits of photosystems II and I. Remmers *et al.* (2017) studied nitrogen-starved batch cultures of *Phaeodactylum tricornutum* to quantify the influence of different occurrences of light intensity on the biomass, TAG and EPA yields. The maximum TAG and EPA contents and maximum biomass concentration levels were established to be self-determining according to the degree of functional light intensity. The lipid yield was reduced at high light intensity levels (>100 μmol$\cdot$m$^{-2}\cdot$s^{-1}). The maximum TAG yield on light (112 mg TAG mol$_{ph}^{-1}$) was found at the lowest light intensity (60 μmol$\cdot$m$^{-2}\cdot$s^{-1}). However, mass balance analysis exposed that the EPA fraction in TAG might originate from photosynthetic membrane lipids.

2.8.2 pH for algal cultivation

Naturally, algal growth caused marked changes in the pH of the culture media. Algal populations cultivated well in artificial culture media of different pH levels between 5 and 12. Furthermore, pH values were temperature dependent, which caused the rate of the pH to change (Dubinsky and Rotem 1974). Nevertheless, certain species effecvtively inhabit more acidic or basic environments. A suitable pH value can effectively complete the cultivation process due to the disruption of a number of cellular methods. The latter is proficient by aerating the culture. The addition of carbon dioxide allows for the ability to correct for increased pH values, which may reach limiting values of up to pH 9 during algal growth in high-density algal culture (Bergstrom *et al.* 2007). After addition of hydrochloric acid or acetic acid or saturated water solution of carbon dioxide stops, the pH value will be continuously increased to 9 or above. Because alkaline metabolites are generated in the growth process of diatoms and a strongly basic environment is adversative to the growth of diatoms. Consequently, the pH value needs to be controlled within an optimum environment for diatom growth. In the cultivation process, pH value range is one of the most advantageous conditions for diatom growth and can result in the best level of diatom cultivation efficiency (Wang 2014).

Leavitt *et al.* (1999) and Wang (2014) stated that algal abundance increased when the pH was lowered from 6.6 to 5.0. It can be expected that if the pH is raised, algal abundance should decrease. Pendersen and Hensen (2003) observed a reduced level of growth in a group of pH tolerant algae in a marine environment when the pH value exceeded 9.5. The most pH-sensitive species decreased in abundance when the pH was raised to 8.8 and stopped growing when the pH exceeded 9.0. In a number of studies, adverse effects on algal abundance were observed when the water pH value was high. Most algae have an optimal range of conditions wherein their growth will increase. Reed and Klugh (1924) compared algal growth in two different habitats, a granite pond of pH 6.2 to 6.8 and a limestone pond of pH 7.6 to 9.2, and studied the biotic characteristics and algal content. The distance between basins was only a few hundred yards. None of algal species found in the limestone pond was found in the granite pond. From this case, it was clear to see how different species of algae have differing tolerances for various pH ranges. Bartual and Gálvez (2002) investigated the effects of pH on the growth and biochemical composition of *Phaeodactylum tricornutum* Bohlin that had been acclimatized to saturated and restricted light conditions. Diatoms were grown in diluted, unbuffered and nutrient enriched batch cultures at various pH values and at subsaturating (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and saturating (150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) light intensities. The results revealed that pH treatments under saturating light did not affect the growth rate. On the other hand, the rate of increase was significantly reduced at pH values of over 8.5 under subsaturating light conditions.

Spilling *et al.* (2013) found that an increase in the pH value could reduce the number of structural lipids in the *Amphiprora* sp. *Phaeodactylum tricornutum* CCAP strain and the *P. tricornutum* TV strain. The effects of the rapid growth process were tested at values ranging from pH7.5 to 10 by adding NaOH. In the CCAP strain, TV strain and *Amphiprora* sp., the total lipid content was reduced by 13, 36 and 47 %, respectively, after increasing the pH over 1 h. For further testing, the *P. tricornutum* CCAP strain has been used to test the pH effect on lipid content during dynamic growth. The pH values were set at 7.5 and 10 and were regulated by the degree of CO_2 influx. In both pH treatments, the growth rate was similar (0.3 day^{-1}). However, in the pH10 treatment, the lipid content was on average 28 % lower than in the pH7.5 treatment. The results propose that regulating the pH value during algae cultivation could be used to enhance the lipid composition in the harvested algal biomass. Scholz (2014) determined that the physiological responses of marine benthic diatoms *Amphora graeffii*, *Navicula gregaria*, *Navicula phyllepta*, *Nitzschia epithemoides* and *Pinnularia ambigua* to pH changes. The growth rates and biochemical compositions were examined at varies pH values (7.25, 7.5, 7.75, 8.0, 8.25, 8.5 and 8.75) for the duration of short-term (6 h) and long-term exposure periods (30 d). While the growth rates of 5 diatom taxa under the short-term conditions were hardly affected, the long-term exposure periods led to decreasing growth rates in all tested species. Gu *et al.* (2017) tested the potential effect of ocean acidification and NH_4-N in *Skeletonema costatum* and *Nitzschia closterium.* The pH decline significantly inhibited growth and NH_4-N uptake of *S. costatum* and *N. closterium*. The maximum uptake rate was greater than the maximum growth rate, implying that with decreasing pH, NH_4-N values, *S. costatum* and *N. closterium* were integrated faster.

Pérez *et al.* (2017) evaluated the mechanism of pH-induced flocculation for *Skeletonema costatum* and *Chaetoceros gracilis*. Both species were tested by acid pH values (2–6) and basic pH values (8–12). Almost a total biomass recovery was extended for the highest pH values (11, 11.5 and 12); whereas a maximum biomass recovery, by around 60%, was achieved for the acid pH values for both species. Subsequently, biomass separation was performed using two different techniques: ultrasound and Soxhlet, to assess oil extraction and yields. Also, chromatographic analysis of the algal oil showed that all fatty acids present were saturated. Thus, pH-induced flocculation could be a promising harvesting method, while the two studied species were determined to be suitable and a potential raw materials for producing lipids.

2.8.3 Temperature for algal cultivation

Among various environmental factors, temperature is an important parameter that affects all biological reactions (Kleinschmidt and Mcmahon 1970).

Temperature stress has a major effect on lipids and the fatty acids profile in algae (Teoh *et al.* 2013). The relationship between the nutritional profile and temperature is of great importance for efficient lipid production from the cultured diatoms. A high growth temperature has been associated with a substantial increase in lipid content in some diatoms (Han *et al.* 2013; Teoh *et al.* 2013). The optimal temperature for phytoplankton cultures is between 20 and 24°C. However, the temperature might diverge with the composition of the species, the strain cultured and the culture medium. Most commonly cultured microalgae tolerate temperatures within a range of 16-27°C. Temperatures lower than 16°C have been found to decelerate growth, whereas those higher than 35°C are lethal for some species. If required, algal cultures can be ventilated by a cold water flow over the culture vessel surface or by controlling the air temperature with refrigerated air-conditioning units.

Subhash *et al.* (2014) designed experiments with a dual mode cultivation, growth phase (GP) and a temperature (25°C, 30°C and 35°C) induced stress phase (SP). GP recorded an enhancement in biomass growth and carbohydrate accumulation, although the SP process at 30°C showed an obvious improvement in lipid productivity levels (total/neutral lipid, 24.5/10.2%). Maximum carbohydrate utilization was observed during SP at 30°C, 25°C and 35°C experiments at 57.8%, 50.6% and 26.9%, respectively, which correlated well with the level of lipid synthesis. Fascinatingly, the neutral lipid content recorded a five-fold increase in exemplifying a level of achievability toward high biodiesel properties. The biodiesel profile at a temperature of 30°C is upheld by higher saturated fatty acid (SFA) to unsaturated fat (USFA) proportions.

Dash *et al.* (2013) studied the taxonomic composition of the phytoplanktons and their abundance for the delineation of their temporal patterns, and studied their basic ecological patterns during the annual cycles of natural hot springs at Atri of Odisha. An analysis of the phytoplankton community composition showed that there were forty species of three different algal classes that were identified as 43% Chlorophyceae, 35% Cyanophyceae and 22% Bacillariophyceae. Wah *et al.* (2015) claimed that growth temperature has a significant influence on both the fatty acid and lipid contents of benthic diatoms. The tropical benthic diatom *Amphora subacutiuscula* was cultivated to test the effects of seven different temperatures on the lipid profile. At

5°C, the quantity and quality of the lipids produced were not only significantly affected, but the biomass production of *A. subacutiuscula* was also affected. Additionally, a temperature of up to 10°C did not influence the distribution of fatty acid methyl ester (FAME). Higher temperatures at up to 23°C benefited the diatoms in terms of growth. This temperature locally isolated benthic diatoms while exhibiting good qualities and quantities of lipids as well as EPA. The results indicated that *A. subacutiuscula* might serve as an alternative source of fish oil. Also, the effects of temperature, salinity, and photosynthetic photon flux density (PPFD) on the growth of *Asteroplanus karianus* were examined by (Shikata *et al.* 2015). The maximum growth rate occurred at 20°C and at a salinity of 20; however, *A. karianus* could not survive at 30°C.

2.9 Diatoms for lipid production

Microalgae have received notable attention as biofuel feedstock because of their high photosynthetic rate that is approximately 50 times higher than terrestrial plants. Thus, microalgae can grow faster than terrestrial crops. They can also accumulate a much greater fraction of oil content in their biomass than conventional crops, e.g., 60% versus 2-3% for soybeans. Furthermore, the major lipid content of microalgae is triglyceride and it is most important for biodiesel production. Microalgal biomass can be converted into biofuel through either thermal or chemical conversion processes (Suali and Sarbatly 2012). Therefore, microalgae remain highly important as a future energy feedstock since they require less land to produce than other commercial crops such as palm oil (Chisti 2008). However, the production costs associated with microalgae as a feedstock are higher when compared to the final yield of the product. Due to limited fossil fuel supplies and global climate change, alternative energy sources must be a concern. Biodiesel manufactured from algae presents great potential in the hopes of replacing petroleum-based transport fuel sustainably. The production of biodiesel from microalgae is a newly developing field and presents an alternative source of biodiesel production with great potential. Microalgae characterize an exceptionally diverse but highly specialized group of microorganisms that are adaptable to various ecological habitats. Microalgal biotechnology seems to retain a high potential for biodiesel production. A significant increase in the lipid content of microalgae is now possible through genetic engineering approaches (Sakthivel 2011).

The fatty acid composition of oil plays a major role in the performance of biodiesel in diesel engines. Saturated FAMEs raise the cloud point, octane number, and enhancement strength, while higher rates of polyunsaturation decreases the cloud point, cetane number and steadiness (Kumar *et al.* 2003). Lipids are the main metabolites of diatoms, e.g., polar lipids, traces of sterols and neutral lipids containing triacylglycerol (TAG), diacylglycerol (DAG) and monoacylglycerol (MAG), with the TAG content typically accounting for more than 60% of the total lipids. Additionally, the fatty acids of diatoms include lauric acid (C12:0) and lignoceric acid (C24:0). Chuecas and Riley (1969) and Wen and Chen (2002) claimed that hexadecanoic acid (C16:0), myristic acid (C14:0), 9-hexadecenoic acid (C16:1) and eicosapentaenoic acid (EPA, C20:5 n-3) are higher in terms of lipid content than other fatty acids. Also, some diatoms contain docosahexaenoic acid (DHA, C22:6 n-3), which is essential for many marine animals. They appear to have a limited capacity for synthesizing long-chain polyunsaturated fatty acids, especially EPA and DHA, that are needed for proper growth and survival (Yongmanitchai and Ward 1991; Renaud *et al.* 1995). Likewise, *Nitzschia laevis* is a potential producer of EPA through lipid-extraction and analysis via gas chromatography (GC) and thin layer chromatography (TLC) (Chen *et al.* 2007). In addition, chemical compounds from microalgae can be incorporated into cosmetics and used as a source of valuable molecules such as polyunsaturated fatty acids, which can be added to infant formulae and nutritional supplements (Milledge 2011). Moreover, Sheehan *et al.* (1998) found that diatoms grown under suitable conditions could deliver up to 60% of their cell mass as triacylglycerols (TAGs). These TAGs can be effortlessly changed into biodiesel through a transesterification response.

Diatoms produce oil intracellularly as safe nourishment material during the vegetative stage of development. Other than high lipid and unsaturated fat substances, there is a wealth of eicosapentaenoic acid, a polyunsaturated fatty acid (PUFA), in diatoms. Alcohol (e.g., methanol) reacts with the triglyceride oils in the transesterification process. Those oils are contained in diatom fats, forming fatty acid alkyl esters (biodiesel) and glycerin (Lebeau and Robert 2003). Johansen *et al.* (1990) claimed that all strains except one of *Chaetoceros muelleri* showed incremental increases in lipid content under nutrient stress. However, by and large, nitrogen stress specifically prompted a higher lipid content. Moreover, Mcginnis *et al.* (1997) cultured *Chaetoceros muelleri* on a thermal gradient plate by relying on two media with a range of specific conductances and assessed growth and neutral lipid accumulation. *C. muelleri* exhibited a very rapid growth rate of at least two doublings per day^{-1} over a broad range of temperature from 20 to 35°C and conductance ranges from 10 to over 60 mS·cm^{-1}. The optimum growth rate approached 4.0 doublings per day^{-1} at 30°C and a conductance of 25 mS·cm^{-1} and yielded large quantities of neutral lipids. Besides, the lipid content in *C. muelleri* appeared to be very susceptible to physiological manipulation. Increased Nile Red relative fluorescence and the gravimetric yield of cellular lipids illustrated that nitrogen deprivation stimulated lipid storage in *C. muelleri*. Dependant upon its high growth rate, tolerance of a broad range of temperatures and specific conductances, and a lot of intracellular lipids, *C. muelleri* may have the potential for utilization as a renewable source of liquid-fuel precursors. Also, when sufficient nutrients are available, proteins are synthesized; however, when nutrients are limited, cell division is inhibited and a greater amount of carbon is available for lipid storage. In many algal species, lipid storage has been induced by the depletion or removal of nitrogen from the culture media (Sukenik and Wahnon 1991).

Natural oils can be changed over to biodiesel by a moderately straightforward refining process called transesterification (Sanjay *et al.* 2013). More than a thousand types of algae including diatoms have been screened to look for high lipid content. It was discovered that under normal conditions, polyunsaturated fatty acid constitutes roughly 25% of the algal mass (Hu *et al.* 2006). This content might vary noticeably between species, and interestingly, the lipid content increases when cells are exposed to unfavorable culture conditions, such as photo-oxidative stress or nutrient starvation. This is due to the shift in lipid metabolism from membrane lipid synthesis to the storage of neutral lipids (Hu *et al.* 2008). The oil extracted from *N. cryptocephala* demonstrated a nearness to palmitic, oleic, palmitoleic and linoleic acids as the fundamental unsaturated fats that can be contrasted with Jatropha oil and rapeseed oil and utilized for biodiesel creation. The free unsaturated fat substance is one of the critical elements for antacid catalyzed transesterification. This is because the free fatty acids respond with the basic impetus to create a cleaner, repressed response and this outcome results in the lessening of the biodiesel yield (Meher *et al.* 2006). Beal *et al.* (2013) used microscopy and chromatography to analyze the progression of the lipid profile and cell structure of the algae cells in the research-scale production pathway for biocrude production. The changes in the lipid content corresponded to the level of cell degradation that was observed by SEM and TEM throughout processing. It is shown that triglyceride content decreased, while diglyceride and free fatty acid contents increased during the processing. Li *et al.* (2017) optimized the growth of *Cyclotella* sp., *Synedra* sp. and *Navicula* sp., via an orthogonal assay on N, P, Si and Fe. Temperature and pH were considered using traditional single-factor tests. Silica had the greatest effect on growth, followed by phosphate and iron. The optimized growth conditions included temperatures of 25 and 30°C and pH values in a range of 7.5-8. Moreover, fatty acid profiling revealed C16:0, C16:1 (*n*−7), C18:0 and C20:5 (*n*−3) as major fatty acids. Additionally, 12 diatom species were cultured in 20 L clear plastic carboys containing 10 L of modified PES media, with initial cell numbers of 1x10^5 cells·mL^{-1}. They were then cultured for 49 days in summer (August–September) and winter (December– January) greenhouses and a walk-in plant incubator with 24°C and a 12:12 light:dark cycle with a light intensity of 122 μmol photons·m^{-2}·s^{-1}. The results indicate that the biomasses, fatty acid compositions and contents differed among the investigated species and the three experimental culturing environments. The diatoms showed various trends for accumulating different kinds of fatty acids. The total lipid content ranged from 30.2 ± 1.59% to 45.1 ± 2.39%. However, the results have successfully indicated that *Amphora bigibba* and *Nitzschia panduriformis* grew quickly in the summer greenhouse and tended to accumulate PUFAs. In contrast, *Cylindrotheca* sp. and *Navicula lyra* tended to accumulate SFAs when cultured in the summer greenhouse. Furthermore, when cultured in the summer greenhouse, *Chaetoceros muelleri* produced the highest total SFA and monoenoic FA contents, and *Seminavis gracilenta* produced the highest total PUFA content. Therefore, this study provides a new method for obtaining the valuable raw materials of DHA, EPA, and other n-3, n-6 FAs from various species of diatoms (Chen 2012). In addition, Wen and Chen (2002) found that the diatom *Nitzschia laevis* is a potential eicosapentaenoic acid (EPA) producer as it could accumulate relatively large amounts of EPA under heterotrophic conditions. The cells were incubated in 500 mL flasks containing 200 mL LDM medium in an orbital shaker with 200 rpm and at 20°C in darkness. Hence, a fed-batch process was developed for the high cell density of this culture for the purposes of enhanced production of EPA because substrate limitation or inhibition can be avoided by maintaining medium substrate concentration at low values during the cultivation process.