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Application note

Application note: A novel low-cost open-source LED system for microalgae cultivation

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ABSTRACT

This application note introduces an Arduino-based LED system useful for cultivation of various microalgae species as well as other plants. The system is based on RGB (APA102C) LEDs connected to an external Arduino microcontroller allowing flexibility via programming, modification, and upgrades. We describe in detail the C functions that produce white light and mixed colors in LED strips as well as the ability to generate intermittent/flashing/pulsing light. The capabilities of the system offer unique applications in industry and research. Our aim is to provide a low-cost and open-source tool in order to improve and promote cultivation of photoautotroph species (bacteria, microalgae or plants) using LEDs.

1. Introduction

The impacts of global warming, increasing prices of commodities, diminution of natural resource and population growth have intensified the research on finding future crops. Microalgae is a promising alternative crop due to their capability to perform photosynthesis, rapid growth, increase yield potential, stress resistance, production of bioactive components and usage as feedstock. Phototrophic cultivation of microalgal cells is carried out in laboratories in an environment of stable and calibrated illumination in clear flasks, culture vessels, bottles, tanks, or alternatively outdoor in order to take advantage of solar energy using tubular or cylindrical photobioreactors, or in open ponds/raceways (Bajpai et al., 2014). Nevertheless, despite considerable research and effort done in the past decades major issues concerning microalgal cultivation systems exist and improving the systems in use are needed. Furthermore, microalga-based applications that have reached industrial scale are relatively scarce, mainly due to high production costs involved, given the current market prices of the products of interest (Carvalho et al., 2011).

The usage of closed bioreactors as an artificial growing system is now established and like some other growing systems a requirement to ensure microalgae growth, the addition of light sources is needed. Light and difficulties associated with its control are a major factor in microalgal cultivation and the bottleneck caused by using conventional light sources can now be addressed by employing innovative technologies such as Light Emitting Diodes (LEDs) (Carvalho et al., 2011; Yam and Hassan, 2005).

The objective of this application note is to develop and promote a low-cost, open-source light solution for microalgae cultivation based on LEDs and the Arduino microcontroller. This note also provides concise background on LEDs, Arduino and photosynthesis as well as the hardware designs and source codes needed. The core topics discussed briefly in the background are LEDs and Arduino microcontrollers (Section 2.1), photosynthesis (Section 2.2) and benefits and disadvantages of LED systems (Section 2.3).

Both the hardware and source codes can be easily modified or scaled up in order to be used in various microalgae cultivation setups and biotechnology research, but can also be applied to other experiments involving plants/non-plant setups which require changeable light regimes. The hardware and source codes section including the LEDs system and an optional cooling solution (fan based) are described in Section 3.1. The source codes include in this paper (different colors, mixing different colors and flashing light) are shown in Section 3.2.

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2. Background

2.1. Light, LEDs and Arduino microcontroller

Visible light is one segment of the electromagnetic radiation spectrum which can be treated as a wave but also as a particle, which is called a photon. Light with a wavelength between 380 and 750 nm has the energy content to sufficiently produce chemical changes in the absorbing molecules while light sources with a wavelength higher than 750 nm have an energy content that is too low to mediate chemical change and the energy absorbed in this range can only be converted to heat. Further, wavelength radiation below 380 nm gives rise to ionizing radiation such as X-rays, which have such a high energy content that it ionizes the molecules it encounters (Kommareddy and Anderson, 2004) and therefore can be quite damaging to living biological systems. The biomaterials that adsorb certain light wavelengths are called pigments and some are essential for photosynthesis. Chlorophylls, carotenoids (carotenes and xanthophylls) and phycobilins are three major classes of photosynthetic pigments in microalgae (Begum et al., 2015). The selection criteria of artificial light sources include high electrical efficiency, low heat dissipation, good reliability, high durability, reasonable compactness, low cost and spectral output falling within the absorption spectrum of the microorganism of interest (Bertling et al., 2006).

Cultivation of microalgae (indoors) require artificial light as it provides better regulation of the photosynthetic photon flux density, photoperiod, and light spectra. Among artificial light sources LEDs are an excellent light source and have gained popularity and flourished for the past few years due to their properties (high efficiency, reliability and low power consumption), decreasing prices and the ability to deliver a low-cost light solution. Conventional light sources (e.g., mercury, metal halide and halogen lamps) are known to be accompanied by large energy losses, which are attributed to high thermal irradiance and often require various auxiliary devices in specific experimental settings such as a set of filters for spectral tuning and shutters to control exposure duration (Teikari et al., 2012).

The recent development of high brightness and addressable (programmable) Red, Green, Blue (RGB) LEDs, driven by a microcontroller have increased their usage in commercial applications. The mix of three single primary colors RGB LEDs is a technique used to generate white light and offers an excellent white light color rendering. By changing the relative intensity of the different color LEDs, it is relatively easy to change the hue of this light source for different applications and with no quantum deficit arising from the Stokes shift loss. However, a relatively complicated external detector and feedback system are required to control the intensity of the light. Furthermore, each LED will degrade at a different rate over a period of time, therefore, certain light intensity ratios emitted by each of these LEDs must be maintained (Yam and Hassan, 2005).

Arduino is an open-source electronics platform based on easy-to-use hardware and software launched in 2005. Arduino platforms are the most well-known and fast spreading electronic prototype developed with the aim to create control devices for projects that are easy to use and inexpensive to acquire. The Arduino Uno is a microcontroller board based on the ATmega328P with 14 digital input/output pins (of which 6 can be used as Pulse Width Modulation (PWM) outputs), 6 analog inputs, 16 MHz quartz crystal, USB connection, power jack, In-Circuit Serial Programming (ICSP) header and a reset button (Arduino—HomePage).

Despite the availability of off-the-shelf microcontrollers and LEDs, their widespread usage in scientific research is limited. There are few scientific articles, technical notes and applications dedicated for the usage of LEDs for plant cultivation and experiments (Shimada and Taniguchi, 2011; Janda et al., 2015; Yeh and Chung, 2009).

2.2. Photosynthesis

Photosynthesis is a photobiochemical process using light energy to produce ATP and NADPH, ultimately consumed in the conversion of CO_2 to organic molecules. Light is a crucial element both in quantity and quality and has to be efficiently transported to the microalgae vicinity to allow photosynthesis. Light is a major factor but, if present in excess, may lead to the formation of harmful reactive oxygen species (ROS) and oxidative stress, phenomena that limit primary productivity. The amount of light energy can influence photosynthesis rates in a number of ways like, availability of the photosynthetic active radiation (PAR), wavelengths, frequency of Light:Dark (L:D) fluctuations, L:D ratio, light acclimated state of the microalgae and the light "history" (continuous or pulsed) (Grobbelaar, 2010).

The first steps of light capture by plants are close to maximum efficiency while later steps are less efficient. It is common to say that the solar energy to biomass conversion will eventually be 1–8% while microalgae seem to absorb light more efficiently, with a photosynthetic efficiency of ca. 20% (Carvalho et al., 2011). Photosynthesis is divided into two parts, light-dependent reactions and light-independent reactions (dark reactions/Calvin cycle) which are considered as the ultimate rate-limiting processes in photosynthesis. It is assumed that dark periods during microalgae growth are necessary but on the other hand, long dark periods (e.g., several hours) will cause microalgae to switch to respiration processes which might lead to a lower growth rate and biomass loss.

As mentioned above light is necessary, and excessive or insufficient light constrains growth and metabolite yields. The photonic flux for photosynthesis (photosynthesis-irradiance curve) can be divided into three categories: (1) light-limited region where the photosynthetic efficiency increases with a rise in irradiance; (2) light saturation area in which the photosynthetic processing capacity of the culture is at maximum and the excessive photonic flux scatters as heat or fluorescence; and (3) photo-inhibition region where increase in light intensity becomes hazardous to the culture which causes a decreased growth rate at the end of the cycle. In photo-inhibition, the photosystem II can be rapidly damaged leading to a decrease in bioproductivity while the photonic flux increases. Microalgae undergo photo-oxidation when the chlorophyll molecule is excited due to high photonic flux, this unstable form can react with oxygen creating an excited oxygen (free radical state). The excited oxygen can cause several unwanted processes, one of which is a reaction with fatty acids which can be harmful to cell and organelle membranes (Carvalho et al., 2011).

Light quality is known to affect microalgal growth as the number of photons in the blue or red wavelengths that can be captured by a chlorophyll molecule depends on the microalgae cellular architecture, pigment composition, and chloroplast arrangement. In microalgal cultures, it has been suggested that at least 5–10% of photons of blue light are required (if red light were used) for other metabolic functions besides photosynthesis; hence, a small amount of white light may be needed in photobioreactors (PBRs), in order to account for such non-photosynthetic needs (Kommareddy and Anderson, 2004). The blue and red wavelengths and their ratio can also affect the biochemical composition, several metabolic pathways and gene expression (Schulze et al., 2014).

A continuous light supply is often used for microalgal cultivation while intermittent/flashing/pulsing light has been investigated to a limited degree due to technical issues; although it is a theoretically suitable alternative and the first experiment using flashing light was done with *Chlorella* by Emerson and Arnold in 1932. From previous works the following facts can be deduced:

(1) the rate of photosynthesis in a system using flashing light is never greater than the rate observed in a system using steady light of the same intensity or the rate in continuous illumination of equal average intensity; (2) the efficiency with which light is received by the algae is utilized tends to be greater in intermittent light than it is in steady light of an intensity equal to that of the light flashes; (3) a precise flash time is not necessary to achieve a considerable increase of efficiency when the incident intensity is high (Kim et al., 2006). More studies showed that flashing light improves microalgae growth and better secondary metabolite production (Kim et al. 2006; Park and Lee, 2001; Xue et al., 2011; Vejrazka et al., 2011; Vejrazka et al., 2012).

2.3. Benefits and disadvantages of LED systems

The rapid development of LED technology as a supplemental control lighting system is driven by consumers and regulatory demand. This high demand is increasing as manufacturing technology has improved LED output, efficiency, availability of wavelengths, spectral composition control, size reduction, and high light output with little radiant heat output (Morrow 2008; Stutte, 2015). LEDs are ideal for use in applications that are subject to frequent on-off cycling and have a better lifetime compared to fluorescent lamps. LEDs have shortened restarting time compared to High-Intensity Discharge (HID) lamps and as they are solid state components they are very resistant to external shock and damage unlike the fragile incandescent light bulb and fluorescent lamps. In addition, their small footprint makes it easy to integrate them into unique forms or printed circuit boards. The superiority of LEDs compared to gas-discharge lamps is of great importance in illumination of photobioreactors as commonly used for microalgae cultivation (Heining and Buchholz, 2015), plant-based biopharmaceuticals production using for example a four-channel dimmable LED system [blue (450 nm), green (525 nm), red (660 nm), and far-red (730 nm)] (Norikane, 2015), microalgal biochemical composition analyses requiring use of specific wavelengths (e.g., increase of pigment and lipids accumulations) (Schulze et al., 2014 and references within), controlling and changing flowering characteristics (Park et al., 2016; Senol et al., 2016), pest management (Ogino et al., 2016), supplemental light source to enhance yield and improving nutritional value of the product being grown (Wojciechowska et al., 2015).

The disadvantages of using LEDs are not many but currently they are more expensive than conventional lighting technologies. Although the initial cost of conventional light sources is less than LEDs, the operational and maintenance costs of LED are lower. LED performance largely depends on correctly managing the heat generated by the LED (especially when working on high intensities). The heat may cause LED chip deterioration and adequate air cooling or use of heat sinking is advised to extend the life of the instrument. Furthermore, LED's must be supplied with the correct voltage and current at a constant flow and can shift color due to age and temperature.

3. Hardware and software development

3.1. LED Arduino hardware

The flexible RGB LED strips used in this setup are sometimes called tri-chips as they have 3 LEDs in one housing, based on each LED having an integrated driver allows the user to control the color and brightness of each LED independently. The combined LED/-driver integrated circuit (IC) strip used in this study is APA102C in a 5050 SMD LED package (dimensions of 5.0 mm × 5.0 mm). The APA102C uses a generic 2-wire standard Serial Peripheral Interface (SPI) for control (with separate data and clock signals) and has no specific timing requirements. Each single RGB LED draws approximately 60 mA when it is set to full brightness, powered by 5V DC. The LED strips of this configuration can be found in 30, 60, or 144 LEDs per meter. The LED strip used in this paper is Adafruit DotStar Digital LED Strip - 60 LEDs although any other LED strip with similar properties would be suitable.

The LEDs are connected to the corresponding pins on the Arduino microcontroller, pin 4 to the DI (data input) and the CI (clock input) is connected to pin 5 while the ground (GND) and 5V are connected to the corresponding pins as shown in Fig. 1.

The usage of LEDs in high brightness mode in an enclosed area like a growth chamber or an incubator may increase the

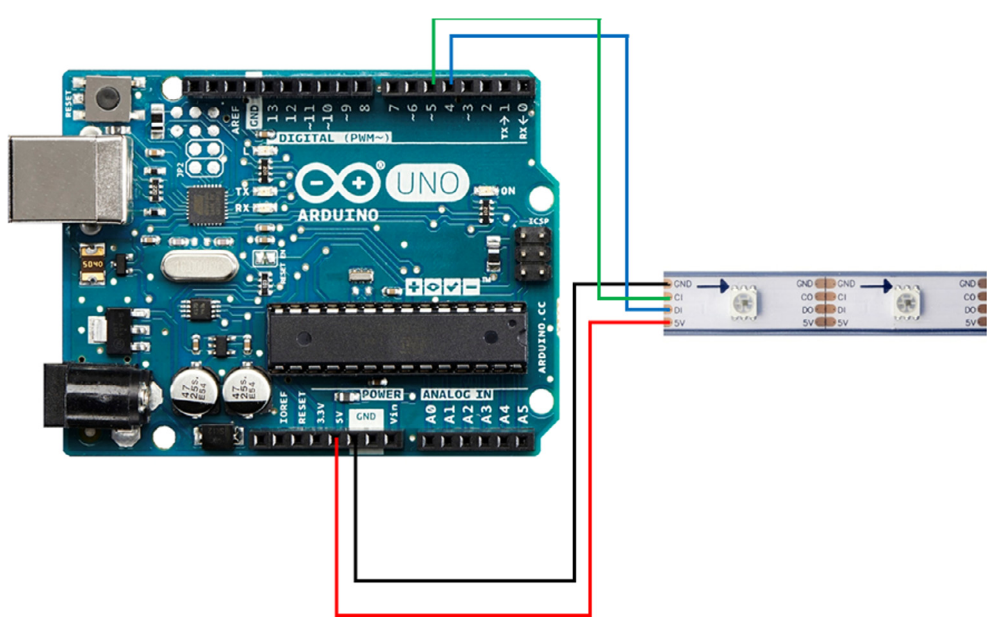

Fig. 1. Arduino-LED strip connecting scheme. A connection scheme between Arduino UNO and Adafruit DotStar Digital LED Strip (Image sources: www.adafruit.com).

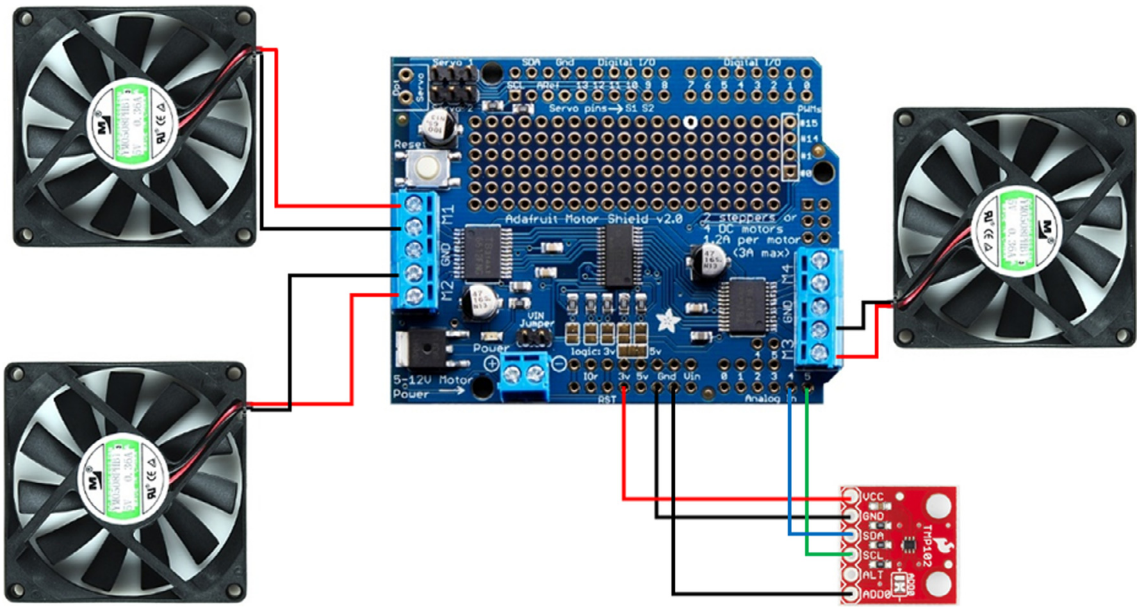

Fig. 2. Motor shield-TMP102-Fans wiring scheme. A connection scheme between Adafruit Motor Shield, 3 5V DC brushless fans and TMP102 temperature sensor (Image sources: www.adafruit.com, www.sparkfun.com).

temperature in the proximity of the culture. In order to decrease the heat accumulation, it is possible to connect fans via a motor shield controlled by a temperature sensor.

The configuration used for cooling includes 3 5V DC brushless fans and Adafruit Motor/Stepper/Servo Shield for Arduino v2 Kit as it has the capability to connect 4 5V DC fans. In a case when more fans are needed this motor shield can support a stackable design. The Arduino motor shield is connected to a low-cost low-power digital temperature sensor (SparkFun Digital Temperature Sensor Breakout - TMP102). The TMP102 ambient temperature sensor is a very simple, accurate, and consume very low-current with a voltage requirement between 1.4 and 3.6 V DC. This small footprint sensor is capable of detecting 0.0625 °C changes between -25 and + 85 °C, with an accuracy of 0.5 °C.

The setup is shown in Fig. 2. The motor shield is situated on top of an Arduino Uno R3 (not shown).

3.2. Arduino source codes

Arduino Integrated Development Environment (IDE) was used to write C codes. A typical Arduino C code consists of two functions that are compiled and linked with a program stub *main ()* into an executable cyclic executive program, the setup and loop. The *setup ()* function marks the actual start of the program with the purpose to set the environment in which the program is run. Similar to the setup function, every Arduino program must have a *loop ()* function and the function is called repeatedly until the board powers off (Purdum, 2015).

The Arduino environment can be extended by using of external libraries and the extra functionality libraries used in this note are SPI.h, Adafruit_DotStar.h and FastLED.h. The SPI.h library allows to communicate with one or more SPI (Serial Peripheral Interface) devices, Adafruit_DotStar.h is a library that allows to control Adafruit Dot Star addressable RGB LEDs and the FastLED.h library allows to address LED strips and pixels in a fast, efficient and easy-to-use way.

The easiest way to achieve white color is shown in source code 1 with detailed comments. In order to achieve white color all three-color RGB LEDs are set to their higher value (255).

Source code 1 - White LEDs:

```
#include <Adafruit_DotStar.h>
#include <SPI.h>

#define NUMPIXELS 51 // Strip length

#define DATAPIN 4 // Data pin to connect to the strip
#define CLOCKPIN 5 // Clock pin to connect to the strip
Adafruit_DotStar strip = Adafruit_DotStar(NUMPIXELS, DATAPIN,
  CLOCKPIN, DOTSTAR_BRG); // The DOTSTAR_BRG describe
  Blue, Red and Green

void setup()
{
  strip.begin(); // Initialize pins for output
  strip.show(); // Initialize all pixels
}

void loop()
{
strip.setBrightness(127); // Max. Brightness (Values 1–255)

  for (int i = 0; i ⩽ NUMPIXELS-1; i = i + 1) {
    strip.setPixelColor(i, 255, 255, 255); // Set each single LED to
  white
    strip.show();
}
}
```

The knowledge relating to the importance of red and blue light in microalgae cultivation is limited. As the LEDs used are tri-chips, each separate LED can be set to emit only red and blue colors beside white. A simple loop was used in order to light the LED strips and is shown in source code 2.

Source code 2 - RGB LEDs:

```
#include <Adafruit_DotStar.h>
#include <SPI.h>

#define NUMPIXELS 51 // Strip length

#define DATAPIN 4 // Data pin to connect to the strip
#define CLOCKPIN 5 // Clock pin to connect to the strip
Adafruit_DotStar strip = Adafruit_DotStar(NUMPIXELS, DATAPIN,
   CLOCKPIN, DOTSTAR_BRG); // the DOTSTAR_BRG describe Blue,
   Red and Green

void setup()
{
  strip.begin(); // Initialize pins for output
  strip.show(); // Initialize all pixels
}

void loop()
{
strip.setBrightness (127); // Max. Brightness (Values 1–255)

  for (int i = 0; i ⩽ NUMPIXELS-3; i = i + 3) {
    strip.setPixelColor(i, 0, 255, 0); // Set single LED to red
    strip.setPixelColor(i + 1, 0, 0, 255); // Set single LED to blue
    strip.setPixelColor(i + 2, 255, 255, 255); // Set single LED to
  white
strip.show();
}
}
```

Flashing LED strips can also be achieved by using FastLED Animation Library (http://fastled.io/). A detailed source code is described for white and RGB flashing light in source code 3 and 4, respectively.

Source code 3 - White flashing LEDs:

```
#include "FastLED.h"

// For led chipsets that are SPI based (four wires - data, clock,
   ground, and power)
#define LED_DT 4 // Data pin to connect to the strip
#define LED_CK 5 // Clock pin to connect to the strip
#define COLOR_ORDER BGR // Color order is BGR for APA102
#define LED_TYPE APA102 // Type of strip used: APA102
#define NUM_LEDS 51 // Strip length

// Define the array of leds
CRGB leds[NUM_LEDS];

void setup() {
    LEDS.addLeds < LED_TYPE, LED_DT, LED_CK,
  COLOR_ORDER > (leds, NUM_LEDS); // Use this for APA102
}

void loop() {
  FastLED.setBrightness(12); // Set brightness to half of the max.
  (max. 255)
  for(int i = 0; i ⩽ NUM_LEDS-1; i = i + 1) {
  leds[i] = CRGB::White; // Set single LED to white
  FastLED.show();
}
  delay(5000); // Turn LEDs to 5 sec.

  for(int i = 0; i ⩽ NUM_LEDS-1; i = i + 1) {
  leds[i] = CRGB::Black; // Set our current dot to black/shut down
   LEDs
  FastLED.show();
}
delay(1000); // Turn LEDs to 1 sec. 1000 = 1 sec.
}
```

Source code 4 - RGB flashing LEDs:

```
#include "FastLED.h"

// For led chipsets that are SPI based (four wires - data, clock,
   ground, and power)
#define LED_DT 4 // Data pin to connect to the strip
#define LED_CK 5 // Clock pin to connect to the strip
#define COLOR_ORDER BGR // Color order is BGR for APA102
#define LED_TYPE APA102 // Type of strip used: APA102
#define NUM_LEDS 51 // Strip length

// Define the array of leds
CRGB leds[NUM_LEDS];

void setup() {
    LEDS.addLeds < LED_TYPE, LED_DT, LED_CK,
  COLOR_ORDER>(leds, NUM_LEDS); // Use this for APA102
}

void loop() {
  FastLED.setBrightness(12); // Set brightness to half of the max.
  (max. 255)
  for(int i = 0; i < NUM_LEDS; i = i + 3) {
  leds[i] = CRGB::White; // Set single LED to white
  leds[i + 1] = CRGB::Red; // Set single LED to red
  leds[i + 2] = CRGB::Blue; // Set single LED to blue
  FastLED.show();
}

  delay(5000); // Turn LEDs to 5 sec.

  for(int i = 0; i < NUM_LEDS; i++) {
  leds[i] = CRGB::Black; // Set our current dot to black/shut down
  FastLED.show();
}

  delay(1000); // Turn LEDs to 1 sec.
}
```

The optional source code for cooling fans controlled by TMP102 temperature sensor is described in source code 5.

Source code 5 – Motor shield-TMP102-Fans:

```
#include <Wire.h>
#include <Adafruit_MotorShield.h>
#include "utility/Adafruit_MS_PWMServoDriver.h"

Adafruit_MotorShield AFMS = Adafruit_MotorShield(); //Create
   the Adafruit_MotorShield object
```

```
// Select port M1, M2 and M3
Adafruit_DCMotor * my1stMotor = AFMS.getMotor(1);
Adafruit_DCMotor * my2ndMotor = AFMS.getMotor(2);
Adafruit_DCMotor * my3rdMotor = AFMS.getMotor(3);
int tmp102Address = 0x48; // Set the address

void setup() {
  Serial.begin(9600); // Set up Serial library at 9600 bps
  AFMS.begin(400); // Connect to the Controller
}

void loop() {
  float Celsius = getTemperature(); // Get the temperature
  Serial.print("Celsius:"); // Output temperature
  Serial.println(Celsius); // Output temperature

  if (Celsius > 25) { // Set fans activation temp. to higher than 25C
  my1stMotor → setSpeed(250); // Sset motor/fan 1 speed
  my1stMotor → run(FORWARD); // Run motor/fan 1 speed

  my2ndMotor → setSpeed(250); // Set motor/fan 2 speed
  my2ndMotor → run(FORWARD); // Run motor/fan 2 speed

  my3rdMotor → setSpeed(250); // Set motor/fan 3 speed
  my3rdMotor → run(FORWARD); // Run motor/fan 3 speed

  delay(120,000); // Run all motors/fans for 2 min. (120 sec.)
}
    else
{
  my1stMotor → run(RELEASE); // Shutdown motor 1
  my2ndMotor → run(RELEASE); // Shutdown motor 2
  my3rdMotor → run(RELEASE); // Shutdown motor 3
}
}

float getTemperature(){ // Acquire temp.
  Wire.requestFrom(tmp102Address,2);

  byte MSB = Wire.read();
  byte LSB = Wire.read();

  //it's a 12bit int, using two's compliment for negative
  int TemperatureSum = ((MSB << 8)|LSB) >> 4;

  float Celsius = TemperatureSum * 0.0625;
  return Celsius;
}
```

4. Conclusions and future trends

In this application note, we described an off-the-shelf, low-cost and open-source LED-Arduino light control system aimed mainly for microalgae cultivation. However, it is recognized that there are other usages and benefits to be gained from LED systems in greenhouses, and other research applications in specific metabolite productivity involving cultivation of higher plants or even photoautotrophic bacteria (Kopsell et al., 2015; Choi et al., 2015; Du et al. 2016). Additionally, the system can be used to examine fundamental and stimulation models of photosystem studies representing wide application domains (Vredenberg et al., 2012). These systems are currently mainly used within the scientific community and have not yet been commercialized.

Today, most laboratories use fluorescent lamps, but LEDs are considered as a viable alternative due to their durability, stability, and low energy consumption. The LEDs provide an outstanding solution and allow excellent operational flexibility as it is easy to control their light quality and quantity (e.g., intensities, wavelength, pulse mode). We provide here a genuinely simple solution with easy steps to implement LEDs in any research. We encourage system modifications as new knowledge is needed in order to optimize light conditions for better microalgae growth and optimization of specific metabolite productions under artificial conditions.

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