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Design and Fabrication of Homogenizer Machine

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Abstract*: Homogenization is any of several processes used to make a mixture of two mutually non-soluble liquids the same throughout. This is achieved by turning one of the liquids into a state consisting of extremely small particles distributed uniformly throughout the other liquid. This paper help the biotechnology to control over the quality of bio-seeds. The quality of seeds and the plants are checked with special DNA process to get the desired results and this process needs Homogenized liquid or pulp of seeds or leaves to be treated in DNA tester and hence our project focus to Develop a machine to homogenize the products without foreign contamination and those must compatible for DNA tester. DNA extraction is a routine procedure used to isolate DNA from the nucleus of cells. This process of testing includes the crushing of leafs, seeds and tissues which further gives result of whether they are useful or not.*

Key words: Homogenization, DNA, AVR, Atmel Studio, SinaProg.

1. INTRODUCTION

Homogenization or homogenization is any of several processes used to make a mixture of two mutually non-soluble liquids the same throughout. This is achieved by turning one of the liquids into a state consisting of extremely small particles distributed uniformly throughout the other liquid.

Many methods have been described for preparing leaf tissue for nucleic acid isolation and like most laboratory protocols, there are as many variations as researchers. Generally, leaf tissue is harvested and processed fresh, frozen and processed cryogenically, or frozen, freeze dried, and then homogenized. Each variation can impact the quality of the DNA, such as the size of the fragments isolated. The protocol used for isolating the DNA will also greatly affect DNA quality. Depending upon the need, harvesting and homogenization are matched for optimal yield.

There are three common methods by which leaf tissue is harvested prior to homogenization. The first involves harvesting leaf tissue followed by freezing. Placing the tissue in a -80°C freezer provides a suitably cold environment that preserves DNA and many proteins, but is unsuitable for preserving RNA. Even at -80°C there is sufficient water activity and nuclease action to degrade RNA, albeit slowly. To harvest leaves and preserve RNA, samples must be frozen rapidly, usually by submersing in liquid nitrogen. To preserve the RNA the samples must be held below -120°C, the glass transition temperature of water. At this temperature all biological activity ceases. The second option of preparing leaf tissue prior to homogenization is to harvest, freeze and then freeze dry the samples. Freeze drying allows for long-term storage of DNA and protein (though not all proteins will remain active), but once again RNA typically doesn't survive the freeze drying process. Freeze drying of leaves removes water which if present can alter the concentration of analyses in extractions buffers. The third options for preparing leaf tissue for disruption is to simply harvest the leaves and homogenize them while they are fresh. With the advent of buffers which preserve DNA and RNA, such as Trizol, disrupting leaf tissue when harvested is often practical

Grinding in Liquid Nitrogen with Mortar & Pestle

One of the most traditional and common methods for harvesting nucleic acids from plants involves grinding leaves in liquid nitrogen with a mortar and pestle. Either the mortar and pestle can be pre-chilled and the grinding performed dry on frozen leaves, or the leaves can be submersed in liquid nitrogen for the grinding. Cryogenic grinding is a very effective technique for taking hard substances, like plant and animal tissues, and turning them into dust. The tough carbohydrates of plant tissues become very fragile at -196°C and easily shatter. The two concerns with cryogenic grinding is that the sample may warm up, and secondly, throughput is very low.

Disruption via Homogenizer: Rotor-Stators and Blenders

For samples which are fresh or freeze dried, homogenization can be attained by shearing leaves with a blade. The simplest bladed homogenizer is a blender, which at times can be completely adequate for disrupting samples. Though efficient for milkshakes, blenders are best used for course shearing while rotor-stators are the preferred tool for efficiently disrupting tissues. Rotor-stators have a spinning circular blade called a rotor inside of a tube with slits, known as the stator. As the blade passes the slits it acts like a fine scissor and shears whatever straddles the slit. . The problem with both blenders and rotor-stators are these homogenizers must be cleaned between use, and in both cases, this may require taking apart the blade assembly.

Bead Beating

Where mortar and pestle and homogenizers fall short in throughput, bead beating makes it up. Bead beating is accomplished using a mixer mill, which is basically a machine that rapidly shakes samples which have been mixed with balls. The balls crash around and effectively shear and crack cells and tissues. Some vortexes will hold multiple tubes making the processing of many samples relatively easy. However, with leaf tissues, it is most practical to use vials or deep well plates and large stainless steel balls. With Vial Sets that use 4 ml polycarbonate vials, the balls are large being 3/8" in diameter. Several hundred milligrams of tissue can be disrupted using a vial. Larger vials can also accommodate up to five grams of leaf tissue. But the most widely used method for homogenizing leaf tissue is to punch leaf holes with a paper punch and drop one disk into a deep well of a micro plate along with a 5/32" stainless steel ball. Using a high throughput homogenizer that holds deep well plates, multiple samples can be processed in minutes.

Beating

Beating a sample using a projectile makes it distinguishable. Most bead beating methods rely on placing a sample and beads in a tube and rapidly shaking them back and forth. Bead beating has been used for years for the disruption of microorganisms, originally using small glass beads and dental amalgamators (i.e., the shakers that dentists use to mix up the components of metal fillings). Bead beating is simply quite effective, though traditionally it was a bottleneck due to the limited number of samples that could be processed. Some labs took it upon themselves to increase the throughput by adapting paint shakers to process samples. At times these were effective, but not totally satisfactory. To remedy this limitation, several companies developed bead beaters (also called mixer mills) that could handle racks of tubes or even microwell plates.

Amalgamators for Tubes: Dental mixers, or amalgamators, have been used to bead beat microorganisms for years. This simple instrument allows a tube to be locked into a little shaking arm which then oscillates rapidly. When bacteria, yeast, or molds are added to the tube with grinding beads, the amalgamator effectively grinds the cells within several minutes.

Limitations – The individual tube bead beaters are rather effective, though throughput is low. For labs running limited samples, this unit might be adequate. Vortexer units are less effective, but hold greater numbers of samples. Depending upon the application, lower lysis efficiency may not matter.

2. OBJECTIVE

- Leaf Crushing should be done in Micro-Tubes.
- Stainless-Steel ball bearing should be used inside Tubes.
- Proposed Machine should be able to crush, mix up to 24 samples simultaneously.
- It should crush all sample types, from soft tissue to soft seeds.
- 1.6 ml micro sealed tubes - eliminating cross contamination.

Figure 1. Uncrushed Leaf in tube

Figure 2. Crushed Leaf in tube

3. WORKING

As Requirements specify the ultimate aim of project, which to produce a pulp inside a tube. The leaf to be crushed, grinding balls and water is added inside the tube. This tubes are mounted Eccentric from the servo motor shaft as shown in figure. By the preprogramed AVR Microcontroller, Servo motor is run clockwise and anti-clockwise for particular time internals at high speed. User specifies the time for which it is to be operated by a numeric keyboard, and is able to view the time on display.

Figure 4.Tube Containing Pulp after Crushing.

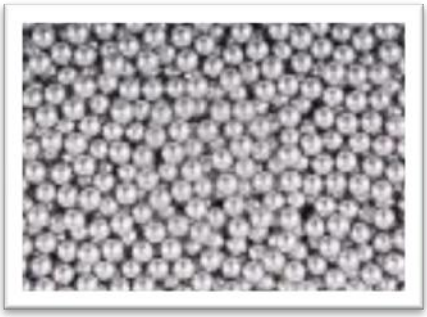

Figure 3. Grinding Balls, 3/8", 440C Stainless Steel

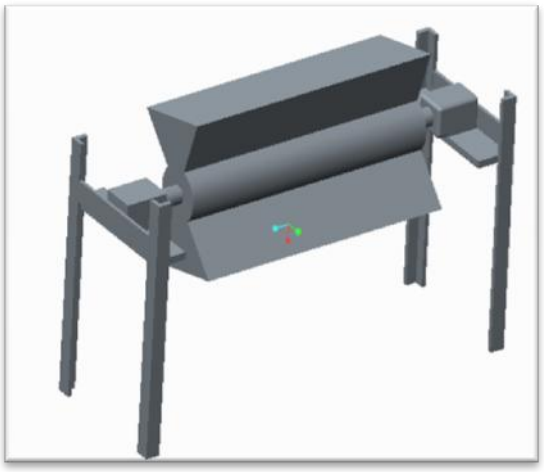

Figure 5. CAD Model of proposed Arrangement.

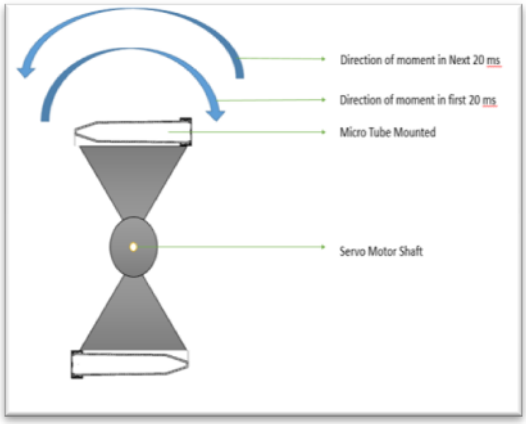

Figure 6: Side View Diagram of Arrangemnt

Motor will be connected through relay and relay will be integrated to Microcontroller.

- Software Required:
  - Atmel Studio 6.0.
  - SinaProg Hex Burner or ISP Burner.
  - USB Drivers.
- Hardware Required:
  - AVR Board
  - AVR USB Programmer.
  - 5V, 1A DC Adapter.
  - Quad Relay Driver.
  - 1 to 1 Connector.
  - Servo Motor
- Programming Language: Embbeded C/C++

AVR Microcontroller

ATmega16 is an 8-bit high performance microcontroller of Atmel's Mega AVR family with low power consumption. Atmega16 is based on enhanced RISC (Reduced Instruction Set Computing, Know more about RISC and CISC Architecture) architecture with 131 powerful instructions. Most of the instructions execute in one machine cycle. Atmega16 can work on a maximum frequency of 16MHz.ATmega16 has 16 KB programmable flash memory, static RAM of 1 KB and EEPROM of 512 Bytes. The endurance cycle of flash memory and EEPROM is 10,000 and 100,000, respectively.ATmega16 is a 40 pin microcontroller. There are 32 I/O (input/output) lines which are divided into four 8-bit ports designated as PORTA, PORTB, PORTC and PORTD.

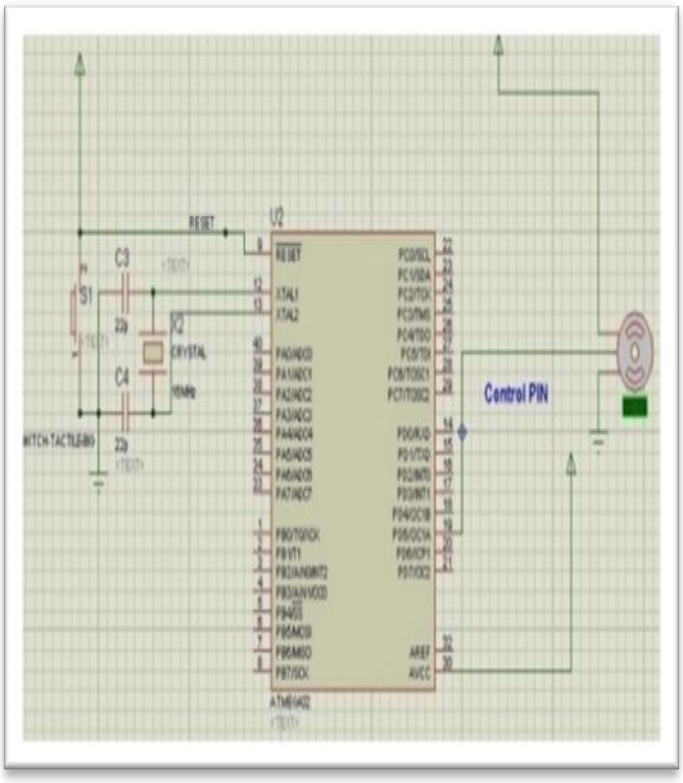

Figure 7. Circuit Diagram of AVR microcontroller

ATmega16 has various in-built peripherals like USART, ADC, Analog Comparator, SPI, JTAG etc. Each I/O pin has an alternative task related to in-built peripherals.

Servo Motor

Servo motors are a type of electromechanical actuators that do not rotate continuously like DC/AC motors. They used to position and hold some object. They are used where continuous rotation is not required so they are not used to drive wheels. In contrast they are used where something is needed to move to particular position and then stopped and hold there. Most common use is to position the rudder of aircrafts and boats etc. Servos can be used effectively here because the rudders do not need to move full 360 degrees nor they require continuous rotation like a wheel. Servos are DC motors with built in gearing and feedback control loop circuitry.

Most servo motors can rotate about 90 to 180 degrees. Some rotate through a full 360 degrees or more. Servos that can rotate 360 degrees are required mainly for building RC helicopters.

Figure 8. Servo motor

Unlike other motors, Servo motors don't require any driver. When a PWM signal is applied to its control pin the, the shaft rotates to a specific angle depending on the duty cycle of the pulse. PWM stands for pulse width modulation. It is basically a modulation technique, in which the width of the carrier pulse is varied in accordance with the analog message signal. As described above, it is commonly used to control the power fed to an electrical device, whether it is a motor, an LED, speakers, etc.

The main thing that is important in the PWM is the duty cycle. It's defined as

$$\% \text{ Duty Cycle} = \frac{\text{On Time}}{(\text{On Time} + \text{Off Time})}$$

4. DESIGN SPECIFICATION FOR SYSTEM

Speed Range:	4.0 to 7.0 m/s (0.05 m/s increments)
Capacity:	24 x 1.6 ml
Noise Level:	<68 db (Low as much as Possible)
Dimensions:	Below 50 x 50 x 80 cm
Weight:	Below 55 lbs / 25kg
Electrical:	100 to 240V, 50Hz

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