3.6 CARBOHYDRATE EXTRACTION

3.6.1 Sample preparation for carbohydrate analysis

3.6.1.1 Acidic extraction

0.3 g of algal sample was treated with 3 mL of 72% H_2SO_4 (v/v) in a 25 mL beaker and then stirred on a magnetic stirrer at 25°C for 30 minutes. Acid treated algal biomass was then transferred in a 500 ml conical flask and diluted with distilled water to maintain a final acid concentration of 2.5%. The reaction flask was then hydrolyzed in an autoclave (30 min, 121°C). After cooling down samples were distributed in two flasks. Sample of one flask was neutralized with NaOH for determination of reducing sugar by DNSA method (Miller, 1959) and Glucose by GOD-POD kit. Sample of other flask was neutralized with sodium carbonate for determination of total carbohydrates by Phenol–Sulphuric acid method (Dubois et al., 1956).

3.6.1.1 Alcoholic extraction

0.1 g of algal sample was treated with 10 mL of 70% ethanol at 80°C in a water bath for 2h. after cooling down it was filtered and the residue was again treated with 70% ethanol at 80°C in water bath for complete extraction of soluble sugars. Both the filtrates were mixed and volume was made upto 100 mL. This solution was used to estimate total soluble carbohydrate and storage glucose estimation.

Note*: Both types of samples were kept under 4°C until used and before using for carbohydrates estimation both types of extracts were centrifuged at 10,000 rpm to remove any type of turbidity.*

3.6.2 Total carbohydrate estimation

Phenol–Sulfuric acid method was used to estimate total carbohydrate (Dubois et al., 1956). According to this method simple sugars and their derivatives gives an orange – yellow colour when treated with phenol and concentrated H_2SO_4 which can be measured by spectrophotometer at 490 nm. For the determination of total carbohydrates in acidic or alcoholic extract 0.1 or 0.2 mL of sample was pipetted out in test tube and volume was made upto 1 mL. A blank was settled with distilled water and standard curve was prepared using standard glucose solution ranging 10µg to 100µg /mL (Fig.3.3). All the test tubes were shaked vigorously after adding 5 mL of 96% H_2SO_4 (v/v) and 1 mL phenol. Then test tubes were incubated in water bath for 20 min at 30°C to developed complete colouration. After cooling of test tubes at room temperature absorbance of samples were noted at 490 nm

against blank. Total carbohydrates, total soluble carbohydrates and total insoluble carbohydrates were calculated as mentioned below.

Total carbohydrate = Total carbohydrate estimated in acidic extracts

Total soluble Carbohydrate = Total carbohydrate estimated in alcoholic extracts

Total insoluble carbohydrate = Total carbohydrate – Total soluble carbohydrate

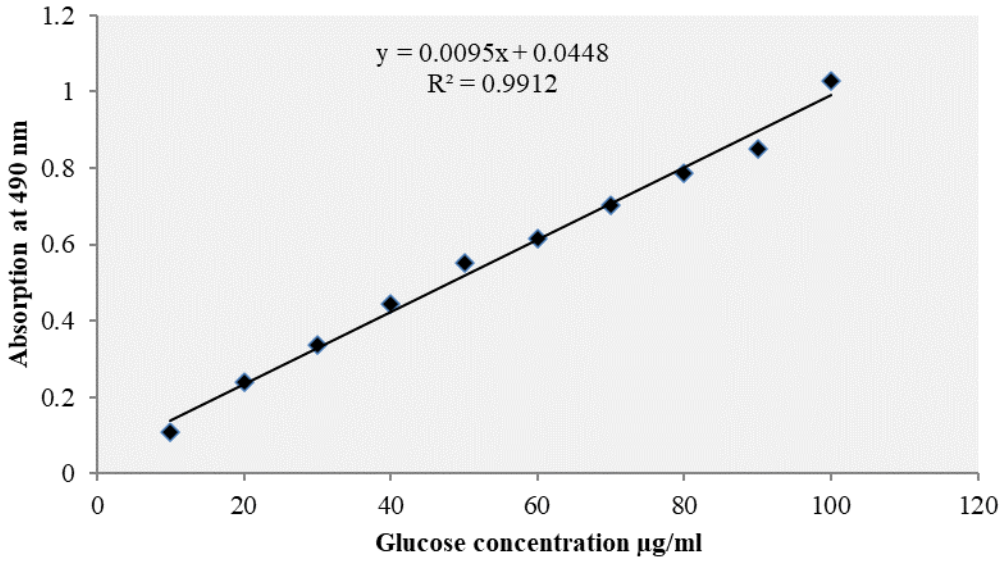

Figure 3.3. Standard curve for total carbohydrate estimation