In jinja a pharmaceutical company having small scale fermentation to produce insulin using genetically modified bacteria however after some time production decline and workers reposted exposure to chemical fumes from a nearby industry observation shows

Bacteria culture exhibited reduced growth and abnormal cell size

Enzyme activity in fermentation tanks declined despite adequate substrate supply

Some bacteria cells showed damaged plasma membrane

Workers exposed to fumes showed fatigue and muscle weakness

DNA analysis received errors in gene replication and protein synthesis

,microscopy showed fewer mitochondria light structure in eukaryotic cells used in control experiment

Further investigation received tat

The nearby factory released cyanided containing compounds

Fermentation temperature fluctuated below optimum level

Substrate concentration was high but enzyme activity

Remained low

Some enzyme showed competitive and none competitive inhibition patterns

Pcr amplification of insulin gene produced inconsistent results

Table 1. cellular enzyme indicator

| parameter | Normal culture | Affected culture |
|---|---|---|
| Enzyme activity (%) | 100 | 50 |
| Bacteria growth rate | High | Low |
| Membrane integrity | 98 | 60 |
| DNA replication accuracy (%) | 99 | 75 |
| Protein synthesis | 100 | 55 |

Table 2. metabolic respiratory indicators.

| parameter | Control | Affected |
|---|---|---|
| Atp production (%) | 100 | 40 |
| Oxygen utilization (%) | 95 | 50 |
| Krebs cycle (%) | 100 | 45 |
| Glycolysis rate | Normal | increased |

*<u>Task</u>*

a) Annalise how enzyme inhibition membrane damage and disruption of cellular respiration affected atp production, protein synthesis and cell survival in both prokaryotic and eukaryotic system

b) Explain how biology and technological approaches could be applied to restore enzyme efficiency to improve recombinant insulin production and protein worker health

**(a) Analysis of how enzyme inhibition, membrane damage and disruption of cellular respiration affected ATP production, protein synthesis and cell survival in prokaryotic and eukaryotic systems**

The decline in insulin production resulted from a combination of enzyme inhibition, membrane damage, cyanide poisoning and temperature fluctuations which interfered with normal cellular metabolism.

**1. Effects of enzyme inhibition**

Enzymes control metabolic reactions in cells. The investigation showed both **competitive and non-competitive inhibition**.

**Competitive inhibition**

- Inhibitor molecules competed with substrate molecules for the active site of enzymes.
- This reduced the rate of enzyme-substrate complex formation.
- Consequently, fewer metabolic products were formed.

**Non-competitive inhibition**

- Inhibitors attached to sites other than the active site.
- This altered the enzyme's shape and reduced its catalytic efficiency.
- Increasing substrate concentration could not overcome this inhibition.

**Effects on ATP production**

- Enzymes involved in glycolysis, the Krebs cycle and oxidative phosphorylation became less effective.
- ATP synthesis declined from 100% to 40% as shown in Table 2.
- Reduced ATP limited energy available for cellular activities.

**Effects on protein synthesis**

- Protein synthesis requires ATP for transcription and translation.
- Reduced ATP supply lowered insulin production and other proteins.

- Protein synthesis decreased from 100% to 55% (Table 1).

**Effects on cell survival**

- Essential metabolic reactions slowed down.
- Growth rate decreased from high to low.
- Some bacterial cells became abnormally large because normal cell division was disrupted.

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**2. Effects of membrane damage**

The plasma membrane regulates movement of substances into and out of cells.

**Observed evidence**

- Membrane integrity reduced from 98% to 60%.
- Some bacterial cells showed damaged plasma membranes.

**Effects on ATP production**

- In bacteria, the plasma membrane contains components of the electron transport chain.
- Damage interfered with proton gradient formation and ATP synthesis.
- Less ATP was generated.

**Effects on protein synthesis**

- Amino acids and other nutrients entered cells less efficiently.
- Waste products accumulated within cells.
- Ribosomes received inadequate raw materials for protein synthesis.

**Effects on cell survival**

- Leakage of ions and metabolites occurred.
- Osmotic balance was disturbed.
- Some cells died due to loss of homeostasis.

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**3. Effects of disruption of cellular respiration by cyanide**

The nearby factory released cyanide-containing compounds.

**Mode of action of cyanide**

- Cyanide inhibits cytochrome oxidase in the electron transport chain.
- Electrons cannot be transferred to oxygen.

- Aerobic respiration stops.

**Effects on ATP production**

- Oxidative phosphorylation was severely reduced.
- ATP production dropped to 40%.
- Oxygen utilization declined from 95% to 50%.
- Krebs cycle activity reduced from 100% to 45%.

**Increased glycolysis**

- Cells compensated by increasing glycolysis.
- Glycolysis produces only small amounts of ATP.
- This explains the increased glycolysis rate observed in affected cultures.

**Effects on protein synthesis**

- Insufficient ATP was available for DNA replication, transcription and translation.
- Recombinant insulin synthesis declined.

**Effects on cell survival**

- Cells experienced energy deficiency.
- Growth and reproduction slowed.
- Prolonged exposure caused cell death.

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**4. Effects on DNA replication and protein synthesis**

DNA analysis revealed errors in gene replication and protein synthesis.

**Causes**

- ATP deficiency reduced efficiency of DNA polymerases and ribosomes.
- Cyanide-induced stress damaged cellular components.
- Temperature fluctuations affected enzyme activity involved in replication.

**Consequences**

- DNA replication accuracy decreased from 99% to 75%.
- Mutations occurred in bacterial cells.
- PCR amplification produced inconsistent results.
- Faulty mRNA molecules were formed.

- Incorrect amino acid sequences reduced insulin quality and quantity.

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**5. Effects of low temperature on enzyme activity**

The fermentation temperature fluctuated below the optimum.

**Effects**

- Enzyme molecules possessed less kinetic energy.
- Frequency of enzyme-substrate collisions decreased.
- Metabolic reactions slowed down.

**Consequences**

- Reduced bacterial growth.
- Lower insulin production.
- Further reduction in ATP generation and protein synthesis.

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**6. Effects on eukaryotic cells**

Microscopy showed fewer mitochondria with altered structure.

**Effects on respiration**

- Mitochondria are the site of aerobic respiration.
- Structural damage reduced ATP production.

**Effects on workers**

- Muscle cells received insufficient ATP.
- Workers developed fatigue and muscle weakness.
- Tissues with high energy demand were most affected.

**Effects on cell survival**

- Reduced ATP impaired active transport, biosynthesis and repair processes.
- Cells became less viable and more susceptible to damage.

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**Conclusion**

Enzyme inhibition, membrane damage, cyanide poisoning and low temperature collectively reduced ATP production, disrupted protein synthesis, lowered DNA replication accuracy and decreased cell survival.

These effects caused poor bacterial growth and reduced recombinant insulin production while also affecting the health of exposed workers.

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**(b) Biological and technological approaches to restore enzyme efficiency, improve recombinant insulin production and protect worker health**

**1. Eliminate cyanide contamination**

**Biological importance**

- Removes inhibition of cytochrome oxidase.
- Restores aerobic respiration and ATP production.

**Technological approaches**

- Install industrial waste treatment systems.
- Use cyanide detoxification technologies.
- Monitor environmental pollutants continuously.
- Enforce environmental regulations.

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**2. Maintain optimum fermentation temperature**

**Biological importance**

- Ensures enzymes function at their optimum rate.
- Increases bacterial growth and insulin synthesis.

**Technological approaches**

- Use automated temperature control systems.
- Install thermostats and temperature sensors.
- Employ computer-controlled bioreactors.

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**3. Optimize enzyme activity**

**Biological approaches**

- Select bacterial strains with more efficient enzymes.
- Use genetically engineered strains resistant to inhibitors.
- Increase production of key metabolic enzymes.

**Technological approaches**

- Immobilize enzymes to improve stability.
- Add enzyme activators where appropriate.
- Remove inhibitors from fermentation media.

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**4. Improve oxygen supply**

**Biological importance**

- Supports aerobic respiration.
- Enhances ATP production.

**Technological approaches**

- Increase aeration and agitation.
- Use oxygen monitoring probes.
- Employ efficient fermenter designs.

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**5. Maintain membrane integrity**

**Biological approaches**

- Supply essential nutrients required for membrane synthesis.
- Use stress-resistant bacterial strains.

**Technological approaches**

- Maintain correct pH and osmotic conditions.
- Prevent exposure to toxic chemicals.

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**6. Improve DNA replication and insulin gene expression**

**Biological approaches**

- Use high-fidelity DNA polymerases.
- Select genetically stable bacterial clones.
- Regularly screen cultures for mutations.

**Technological approaches**

- Optimize PCR conditions.

- Maintain sterile conditions to avoid contamination.
- Use molecular markers for quality control.

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**7. Increase ATP production**

**Biological approaches**

- Enhance respiratory enzyme activity.
- Use strains with efficient metabolic pathways.

**Technological approaches**

- Ensure adequate oxygen supply.
- Provide optimal nutrient concentrations.
- Prevent respiratory poisons from entering the system.

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**8. Protect workers' health**

**Biological importance**

- Prevents cyanide poisoning and respiratory disorders.

**Technological approaches**

- Provide personal protective equipment (PPE).
- Install ventilation and fume extraction systems.
- Conduct routine medical examinations.
- Monitor air quality continuously.
- Train workers on chemical safety procedures.

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**Overall conclusion**

The reduced insulin production was mainly caused by cyanide-induced inhibition of cellular respiration, enzyme inhibition, membrane damage and low fermentation temperatures. These factors reduced ATP production, protein synthesis, DNA replication accuracy and cell survival. Restoring optimal fermentation conditions, removing cyanide contamination, improving enzyme efficiency, enhancing oxygen supply and protecting workers from toxic fumes would increase recombinant insulin production and improve worker health.

**(a) Analyse how enzyme inhibition, membrane damage and disruption of cellular respiration affected ATP production, protein synthesis and cell survival in both prokaryotic and eukaryotic systems.**

The reduction in recombinant insulin production was caused by enzyme inhibition, membrane damage and disruption of cellular respiration resulting from cyanide pollution and low fermentation temperatures. Enzyme activity declined to 50% despite high substrate concentration, indicating both competitive and non-competitive inhibition. Competitive inhibitors competed with substrates for active sites while non-competitive inhibitors altered enzyme structure, reducing catalytic efficiency. Consequently, metabolic reactions involved in ATP generation, DNA replication and protein synthesis slowed down. Low fermentation temperatures further reduced kinetic energy and enzyme-substrate collisions, lowering enzyme activity and bacterial growth.

Cellular respiration was severely affected by cyanide compounds released from the nearby factory. Cyanide inhibits cytochrome oxidase in the electron transport chain, preventing transfer of electrons to oxygen. As a result, oxidative phosphorylation was reduced, leading to a decrease in ATP production from 100% to 40%, oxygen utilization from 95% to 50% and Krebs cycle activity from 100% to 45%. The increased glycolysis rate observed in affected cultures indicates that cells attempted to compensate for reduced aerobic respiration; however, glycolysis yields much less ATP. Since ATP provides energy for biosynthetic processes, reduced ATP availability led to a decline in protein synthesis from 100% to 55%, thereby lowering insulin production.

Membrane integrity decreased from 98% to 60%, indicating severe plasma membrane damage. In prokaryotes, the plasma membrane contains respiratory enzymes and electron transport chains. Damage to this membrane interfered with ATP synthesis, nutrient uptake and waste removal. Leakage of cellular contents and loss of ion gradients disrupted homeostasis and reduced cell survival. This explains the low bacterial growth rate and abnormal cell size observed in the affected cultures. Reduced ATP and nutrient availability also impaired ribosomal activity, resulting in decreased protein synthesis and lower insulin production.

DNA replication accuracy declined from 99% to 75%, leading to mutations and errors during gene expression. Since the insulin gene is carried on recombinant DNA, replication errors produced faulty templates for transcription and translation. Consequently, protein synthesis became less efficient and PCR amplification of the insulin gene produced inconsistent results. Reduced ATP availability further impaired DNA polymerase and ribosome function, worsening replication and translation errors.

In eukaryotic cells used in control experiments, microscopy revealed fewer mitochondria with altered structure. Since mitochondria are the site of aerobic respiration, their damage reduced ATP generation. Low ATP levels impaired protein synthesis, active transport and other metabolic activities essential for cell survival. In workers exposed to cyanide fumes, reduced ATP production in muscle cells resulted in fatigue and muscle weakness because muscle contraction requires large amounts of energy. Therefore, enzyme inhibition, membrane damage and cyanide-induced respiratory disruption collectively reduced ATP production, impaired protein synthesis, lowered cell survival and decreased recombinant insulin production in both prokaryotic and eukaryotic systems.

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**(b) Explain how biological and technological approaches could be applied to restore enzyme efficiency, improve recombinant insulin production and protect worker health.**

Several biological and technological measures can be applied to restore enzyme efficiency and improve insulin production. Firstly, cyanide pollution should be eliminated through treatment of industrial waste and strict environmental monitoring. Removal of cyanide would restore electron transport chain activity, increase oxygen utilization and improve ATP production. This would enhance protein synthesis and bacterial growth.

Secondly, fermentation temperature should be maintained at the optimum level using thermostatically controlled bioreactors. Optimum temperature increases enzyme-substrate collisions and maximizes enzyme activity, thereby improving metabolic reactions involved in ATP production and insulin synthesis. Automated sensors and computerized control systems can be used to maintain constant fermentation con
ditions.

Thirdly, enzyme inhibition can be reduced by removing inhibitory substances from the fermentation medium and adjusting substrate concentrations appropriately. Competitive inhibition may be overcome by increasing substrate concentration, while non-competitive inhibition requires removal of the inhibitor. Genetic engineering can also be used to develop bacterial strains with enzymes that are resistant to inhibitors and environmental stress.

Adequate aeration and agitation should be provided to ensure sufficient oxygen supply for aerobic respiration. Increased oxygen availability enhances ATP production through oxidative phosphorylation and supports rapid bacterial growth. Nutrient composition should also be optimized to provide amino acids, minerals and energy sources necessary for protein synthesis and membrane repair.

Genetic stability of the insulin-producing bacteria can be improved through the use of high-fidelity DNA polymerases, regular screening for mutations and optimization of PCR conditions. This will improve DNA replication accuracy, ensure proper expression of the insulin gene and increase insulin yield. Molecular techniques such as DNA sequencing and recombinant DNA quality control can be used to detect and eliminate defective clones.

To restore membrane integrity, optimum pH and osmotic conditions should be maintained and toxic chemicals prevented from entering the fermentation system. Healthy membranes improve nutrient uptake, waste removal and ATP generation in bacterial cells.

Worker health can be protected by installing effective ventilation and fume extraction systems, providing personal protective equipment such as respirators, gloves and masks, conducting routine medical examinations and continuously monitoring air quality. Workers exposed to cyanide should receive immediate medical treatment and safety training. These measures reduce exposure to toxic fumes and prevent respiratory and metabolic disorders.

In conclusion, elimination of cyanide pollution, maintenance of optimum fermentation conditions, reduction of enzyme inhibition, improvement of oxygen supply, enhancement of genetic stability and protection of workers would restore enzyme efficiency, increase ATP production, improve protein synthesis and ultimately increase recombinant insulin production.

Reall ans

**(a) Analyse how enzyme inhibition, membrane damage and disruption of cellular respiration affected ATP production, protein synthesis and cell survival in both prokaryotic and eukaryotic systems. (15 marks)**

**Enzyme inhibition**

**Enzyme inhibition reduced enzyme activity from 100% to 50%, slowing down metabolic reactions in both prokaryotic and eukaryotic cells. Competitive inhibitors competed with substrates for the active site, while non-competitive inhibitors changed the shape of enzymes and reduced their activity. As a result, less ATP was produced and fewer substances needed for growth and protein synthesis were formed. This contributed to the reduction in protein synthesis from 100% to 55% and lowered cell survival.**

**Membrane damage**

**Membrane integrity decreased from 98% to 60%, showing that many cells had damaged plasma membranes. In both prokaryotic and eukaryotic cells, membrane damage caused leakage of cell contents and reduced nutrient uptake. Since nutrients are required for respiration and protein synthesis, ATP production decreased and normal cell activities were affected. The loss of membrane integrity also reduced cell survival because cells could no longer maintain a stable internal environment.**

**Disruption of cellular respiration**

**Cyanide disrupted cellular respiration by inhibiting enzymes in the electron transport chain. This reduced oxygen utilisation from 95% to 50%, ATP production from 100% to 40% and Krebs cycle activity from 100% to 45%. In eukaryotic cells, damaged mitochondria further reduced ATP production, while in prokaryotic cells respiration occurring on the cell membrane was affected. Due to the low ATP supply, protein synthesis decreased because transcription and translation require energy. Cells responded by increasing glycolysis, but this could not produce enough ATP to support normal growth and survival.**

**Effect on protein synthesis and cell survival**

**The reduction in ATP production and enzyme activity caused protein synthesis to decrease from 100% to 55%. DNA replication accuracy also fell from 99% to 75%, resulting in errors during protein formation. Consequently, fewer functional proteins, including insulin, were produced. The combined effects of low ATP, membrane damage and reduced protein synthesis led to a low bacterial growth rate, abnormal cell size and reduced survival of both prokaryotic and eukaryotic cells.**

**Conclusion**

**Therefore, enzyme inhibition, membrane damage and disruption of cellular respiration reduced ATP production, lowered protein synthesis and decreased cell survival in both prokaryotic and eukaryotic systems. This ultimately caused the decline in insulin production.**

**(b) Explain how biological and technological approaches could be applied to restore enzyme efficiency, improve recombinant insulin production and protect worker health. (15 marks)**

**Biological approaches**

Enzyme efficiency can be restored by maintaining optimum conditions for enzyme activity. The fermentation temperature should be kept at the optimum level because low temperatures reduce enzyme activity and slow metabolic reactions. The pH of the fermentation medium should also be monitored and maintained at the optimum range to ensure enzymes function effectively. In addition, toxic substances such as cyanide should be removed from the culture environment because they inhibit enzymes involved in cellular respiration and ATP production. Healthy genetically modified bacterial strains with stable insulin genes can also be selected to ensure efficient protein synthesis and high insulin yield.

**Technological approaches**

Modern fermentation technology can be used to improve enzyme efficiency and insulin production. Automated fermenters fitted with temperature, pH and oxygen sensors can continuously monitor and control culture conditions. Bioreactors can be equipped with cooling and heating systems to prevent temperature fluctuations. Air filtration and purification systems can be installed to prevent contamination by industrial pollutants such as cyanide-containing compounds. Enzyme activity and substrate concentrations can also be monitored using biosensors to detect problems early and allow corrective action.

**Improving recombinant insulin production**

Recombinant insulin production can be improved by maintaining healthy bacterial cultures under optimum conditions for growth and protein synthesis. Adequate oxygen supply, proper nutrient concentrations and stable environmental conditions will increase ATP production and support insulin synthesis. Molecular techniques such as PCR and DNA sequencing can be used to identify and correct mutations in the insulin gene, ensuring that functional insulin is produced. Regular quality control tests should also be carried out to monitor insulin yield and product quality.

**Protecting worker health**

Workers can be protected from harmful chemical fumes by installing proper ventilation and air-extraction systems in the workplace. Personal protective equipment (PPE) such as masks, gloves and protective clothing should be provided and used consistently. Regular monitoring of air quality should be conducted to detect toxic gases such as cyanide before they reach dangerous levels. Workers should also undergo routine medical examinations to identify early signs of chemical exposure, such as fatigue and muscle weakness. Training on chemical safety and emergency response procedures should be provided to reduce occupational health risks.

**Conclusion**

Therefore, restoring optimum enzyme conditions, using advanced fermentation technology, maintaining genetically stable bacterial cultures and implementing workplace safety measures would improve enzyme efficiency, increase recombinant insulin production and protect workers from the harmful effects of chemical exposure.