CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com Research Article

MICROBIOLOGICAL EVALUATION OF NITROGEN GAS IN PHARMACEUTICAL INDUSTRY

Gunaseelan. R[1*] and Viswanathan. T [2]

[1] Research and Development Centre, Bharathiar University, Coimbatore - 641046, India.

[2] Associate Professor, Department of Microbiology, L.R.G Govt. Arts College for Women, Tirupur-641 604.

Abstract:

Nitrogen gases are used at different stages of the pharmaceutical manufacturing process for several applications. Nitrogen gas sampling for microorganisms plays vital part in the contamination control. The aim of the work was to evaluate the aerobic and anaerobic microbial count in nitrogen gas during drug manufacturing process. The Nitrogen samples were collected at two different sites about 1000 liters of air sampled by using M-Air- T air sampler and petri plates containing soya bean casein digest agar used for sampling. The processed plates were incubated for aerobic microbial count at 20-25° C for 3 days for fungal count, followed by 30-35° C for 3-5 days for bacterial count. Incubated anaerobic microbial count at 30-35°C for 3 days using anaerobic jar and anaerobic gas pack. After incubation the plates were evaluated for microbial count. The results observed during the study met the acceptance limits as per the ISPE guideline, the microbial count for non-sterile applications limit is to be established based on the product bioburden limits. Typical level NMT 5 cfu/m^3 and for sterile applications as per the viable particle requirements for grade area where the product is exposed to the compressed gas (eg.Grade A, Grade A/B, Grade B or Grade C). The pharmaceutical manufacturing industries are supposed to set up the limit and acceptance criteria based on the periodic evaluation.

Keywords: *Nitrogen gas, Aerobic, Anaerobic microbial count, Sterile, Non-sterile Pharmaceutical industry*

Corresponding Author:

Gunaseelan. R

Research Scholar

Department of Microbiology

Bharathiyar University,

Coimbatore,

Tamilnadu – 641046, India.

E-mail: cvramguns@gmail.com

Ph: + 91 422-2428100, Fax: + 91 422-2422387

QR code

*Please cite this article in press as Gunaseelan.R and Viswanathan.T., **Microbiological Evaluation of Nitrogen Gas in Pharmaceutical Industry** , Indo Am. J. P. Sci, 2017; 4(11).*

INTRODUCTION:

Nitrogen gas plays a significant role in the pharmaceutical industry, with a broad range of applications such as transfer, purging, blanketing, drug manufacturing, aseptic packaging, analytical testing, filtration, separation and purification [7]. Nitrogen gas is an inert gas, which protect from aerobic microbial contamination in the manufactured drug and increase their shelf life of drug. The presence of aerobic and anaerobic microorganisms in nonsterile and sterile preparations may have the potential to decrease or inactivate the therapeutic activity of the drug and which in turn adversely affect the health of the patient [10].

Aerobic bacterium is an organism which can survive and grow in an oxygenated environment. Anaerobic bacteria are organisms that are capable of surviving and growing in an atmosphere presence of little or no oxygen. Anaerobic bacteria can be further classified based their relationship to oxygen obligate, aerotolerant or facultative anaerobes. Aerobic and anaerobic are found in a variety of environments ranging from soil, water, humans and animals [4].

Various methods have been stated for the assessment of Nitrogen gas analysis for microbial contamination using M-Air-T sampler (Millipore), Active air sampler and using purging in sterile liquid media and test by filtration technique. In recent years, M-Air-T sampler / Active air sampler have been developed to evaluate the microbial count from compressed gas monitoring. In the present study, M-Air-T sampler has been adopted to evaluate the microbial contamination in nitrogen gas.

The present work aim was to evaluate the aerobic and anaerobic microbial count in nitrogen gas during drug manufacturing process. Rationale behind the work was, to control aerobic and anaerobic microbial count throughout manufacturing process.

MATERIALS AND METHODS:

Chemicals and reagents

Pre-incubated Soyabean casein digest agar were supplied by Himedia. Culture *Staphylococcus aureus* ATCC 6538 *Pseudomonas aeruginosa* ATCC 9027 *Escherichia coli ATCC 8739 Candida albicans* ATCC 10231 *Aspergillus niger* ATCC 16404, *Clostridium sporogens* ATCC19404 obtained from American type culture collection (ATCC). Anaerogas pack and indicator obtained from Himedia.

Instrumentation

M Air T (Air sampler) Millipore, Incubators make Thermolab, autoclave makes machine fabric, laminar airflow unit make Esco,

Preparation of Plates:

Prepared Soya bean Casein Digest Agar plates for aerobic microbial count and anaerobic microbial count. The media plates were subjected for growth promotion test with above mentioned culture aerobic and anaerobic condition.

Transfer of Plates to the Area:

Covered the plates with aluminum foil in a stack of 10 plates and placed the plates in the plate carrier stand. Transferred the plates to respective areas. After sample collection the plates are again wrapped the plates with aluminum foil and transferred the plates back to the laboratory. Unwrapped and incubated the plates in the anaerobic jar.

Sample Location and Collection:

Sample has been collected from generation point and user point.

Nitrogen Gas Sampling:

Sampler head and connection tubes were sterilized at 121°C for 30minuts. Transfer all the required material to the sampling location. Opened sampler head and place media cassette and placed pressure reducer and connected to sampling port, 1000 litre of Air Sampled M-Air- T air sampler make Millipore[11]. The sample inlet is connected to the nitrogen gas line and air is directed over an agar plate. The method works by compressed gas under reduced pressure (called “partial flow”) is forced over the surface of an agar plate. The micro-organisms, due to their weight, are flung into the agar surface, whereas the air molecules are deflected. Appropriately incubated, they grow into colonies, which are counted on the assumption that one microorganism gives rise to one colony [5].

The sampling time should be sufficient in order to sample one cubic meter of the agar. After sampling, the agar plate is removed and incubated within a microbiology laboratory. At the end of incubation, the agar is examined for colony forming units. If colony forming units are recovered, these should be assessed against the appropriate limit. It is good practice to identify the contaminants recovered; the identification may provide important information to help determine the origin of the micro flora [9].

The approaches identify a risk, rate the level of the risk, and then develop a plan to reduce, control, and monitor the risk. For example, the monitoring of the risk will help to regulate the occurrence, locations, and level for environmental monitoring [6].

Negative Control of Environmental Monitoring Petri Plates:
The pre-incubated Soya bean casein digest agar plates of the same lot shall be carried out all the way and incubated along with the sampled media plates as a negative control.

Incubation:
The processed plates were transferred to microbiology department and incubated for aerobic microbial count at 20-25° C for 3 days fungal count, followed by 30-35° C for 3-5 days for bacterial count [8]. Incubated anaerobic microbial count at 30-35°C for 3 days aerobic and anaerobic condition separately. After incubation the plates were removed from the incubator to process the recording the result.

RESULTS:
The nitrogen sampling study performed for one year in the one month frequency to cover entire seasonal changes. However one month data has been tabulated and <1cfu/ m^3 counts were observed in Fungal count, Aerobic microbial count for bacteria and anaerobic microbial count. The data's are presented in the Table 1 and Table 2.

Table 1: Aerobic and Anaerobic Microbial Count of Nitrogen Sampling (Site 1)

S. No	Date of sampling	Sampling point	Fungal count	Aerobic microbial count (Bacterial count)	Anaerobic microbial count
1	14/04/2015	NA – 01 (Generation point)	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
2	14/04/2015	NA-02 (User point)	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
3	14/04/2015	NA-03 (User point)	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
4	14/04/2015	NA-04 (User point)	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
5	14/04/2015	NA-05 (User point)	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
6	14/04/2015	NA-06 (User point)	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
7	14/04/2015	NA-07 (User point)	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3

Table 2: Aerobic and Anaerobic Microbial Count of Nitrogen Sampling (Site 2)

S. No	Date of sampling	Sampling point	Fungal count	Aerobic microbial count (Bacterial count)	Anaerobic microbial count
1	22/11/2015	PRS P – II, Nitrogen Outlet	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
2	22/11/2015	RVD - 301	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
3	22/11/2015	RVD - 302	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
4	22/11/2015	RVD - 303	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
5	22/11/2015	N2 Generation - Utility	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
6	22/11/2015	ANF - 301	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
7	22/11/2015	ANF - 302	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
8	22/11/2015	ANF - 303	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
9	22/11/2015	CF-201	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3
10	22/11/2015	CF-202	< 1 cfu /m^3	< 1 cfu /m^3	< 1 cfu /m^3

DISCUSSION:

During the one year evaluation of nitrogen sampling in drug manufacturing process the Aerobic and Anaerobic Microbial Count in site 1 and 2 no growths were observed.

Sterility is achieved through the use of a bacterial retentive membrane filter (0.2 µm pore size) [7]. A sterile-filtered gas is required where a gas contacts a sterilized component or material. It is important that the sterilizing grade filter is maintained dry for condensate in a gas filter will most probably cause blockage or lead to microbial contamination. Risks of condensate are controlled by heating and use of hydrophobic filters (to prevent moisture residues in a gas supply system) [2].

All product get in touch with nitrogen gases should be assessed aerobic and anaerobic microbial contamination. A sampling plan should consider and adapt to increased or reduced production schedules, seasonal changes, equipment changes and modifications, replacement of hardware or filters.

AS per the ISPE Good Practice Guide - Process Gases, the microbial count for non-sterile applications limit is to be established based on the product bioburden limits. Typical level NMT 5 cfu/m^3 [4] and for sterile applications as per the viable particle requirements for grade area where the product is exposed to the compressed gas (eg.Grade A, Grade A/B, Grade B or Grade C) for aerobic microbial count. The result during the above mentioned study met the acceptance limits as per the ISPE guideline requirement [3].

CONCLUSION:

The assessment of nitrogen gas is an important part of quality control within pharmaceutical organizations whether that assessment is for chemical impurities, particulates, or microorganisms [7]. This paper has assessed the appropriate standards for nitrogen gas focused on microbiological sampling. In doing so, the important features of air sampling have been raised together with the factors that can lead to microbial contamination occurring. These aspects should be built into a biocontamination control program. The pharmaceutical manufacturing industries should establish the limit and acceptance criteria based on the trend.

ACKNOWLEDGEMENT:

The authors would like to express the gratitude to the management of Sequent Research limited and Bharathiyar University, for providing necessary support to carry out the work.

REFERENCES:

1.David J. Hentges (1996), Chapter 17:Anaerobes: General Characteristics University of Texas Medical Branch at Galveston;. [ONLINE] Available at: https://www.ncbi.nlm.nih.gov/books/NBK7638/

2.FDA (2004) Guidance for Industry Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice, U.S. Department of Health and Human Services Food and Drug Administration, Rockville, MD, USA

3.IPSE Good Practice Guide: Process Gases, 2011, 5. IPSE, USA, pp81-86 [ONLINE] Available at: https://www.ispe.org/publications/guidance-documents/good-practice-guide-process-gases [Accessed 12 July 2015].

4.ISO 8573-1:2010 Compressed Air Contaminants and Purity Classes, International Standards Organization, Geneva, Switzerland.

5.ISO 8573-7:2003 Test method for viable microbiological contaminant content, International Standards Organization, Geneva, Switzerland

6.Sandle, T. (2013). Contamination Control Risk Assessment in Masden, R.E. and Moldenhauer, J. (Eds.) Contamination Control in Healthcare Product Manufacturing, Volume 1, DHI Publishing, River Grove: USA, pp423-474

7.Sandle, T. (2015). Microbiological Assessment of Compressed Gases in Pharmaceutical Facilities | IVT

8.Sandle, T. (2014) Examination of the Order of Incubation for the Recovery of Bacteria and Fungi from Pharmaceutical Cleanrooms, International Journal of Pharmaceutical Compounding, 18 (3): 242 – 247

9. United states pharmacopoeia (USP39 NF 34),General Chapters: <1116> Microbiological Control and Monitoring Of Aseptic Processing Environments.

10.United states pharmacopoeia (USP39 NF 34), Chapter <1111> Microbiological examination of nonsterile products: acceptance criteria for pharmaceutical preparations and substances for pharmaceutical use.

11.User Guide M Air T® Compressed Gas and Air Sampling Kit, Millipore, P36483, Rev. –, 10/02.