WASTEWATER GENERATION BY SEAFOOD PROCESSING PLANTS LOCATED IN AND AROUND AROOR, KERALA, INDIA: STATUS, CHARACTERIZATION AND TREATMENT USING STRINGED BED SUSPENDED BIOREACTOR

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By

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Wastewater Generation by Seafood Processing Plants Located in and Around Aroor, Kerala, India: Status, Characterization and Treatment Using Stringed Bed Suspended Bioreactor

Ph.D. Thesis under the Faculty of Environmental Studies

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Certificate

This is to certify that the thesis entitled "**WASTEWATER GENERATION BY SEAFOOD PROCESSING PLANTS LOCATED IN AND AROUND AROOR, KERALA, INDIA: STATUS, CHARACTERIZATION AND TREATMENT USING STRINGED BED SUSPENDED BIOREACTOR**", is an authentic work carried out by Smt. Sherly Thomas, under my supervision and guidance at the School of Environmental Studies, Cochin University of Science and Technology, in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Environmental Management, under the faculty of Environmental Studies, Cochin University of Science and Technology, and that no part of this work has previously formed the basis for the award of any other degree, diploma, associateship, fellowship or any other similar title or recognition.

Dr. M. V. Harindranathan Nair
Supervising Guide

Declaration

I hereby declare that the thesis entitled "**WASTEWATER GENERATION BY SEAFOOD PROCESSING PLANTS LOCATED IN AND AROUND AROOR, KERALA, INDIA: STATUS, CHARACTERIZATION AND TREATMENT USING STRINGED BED SUSPENDED BIOREACTOR**" is a genuine record of the research work carried out by me under the supervision and guidance of **Dr. M.V. Hrindranathan Nair**, Associate Professor, School of Environmental Studies, Cochin University of Science and Technology. The work presented in this thesis has not been submitted for any other degree or diploma earlier.

Kochi - 22 **Sherly Thomas**

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Sherly Thomas

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List of Abbreviations

AMEC	–	Arctic Military Environmental Cooperation
ANAMMOX	–	Anaerobic Ammonia Oxidation
AO	–	Ammonia Oxidation
AOB	–	Ammonia Oxidizing Bacteria
APHA	–	American Public Health Association
ASP	–	Activated Sludge Process
ATS	–	Aerobic Treatment System
B F	–	Block Frozen
BDL	–	Below Detection Limit
BGLB	–	Brilliant Green Lactose Bile Broth
BIS	–	Bureau of Indian Standards
BNR	–	Biological Nitrogen Removal
BOD	–	Biochemical Oxygen Demand
CANON	–	Completely Autotrophic Nitrogen removal Over Nitrite
CETP	–	Common Effluent Treatment Plants
CFFT	–	Cuttlefish Filleting
CFTN	–	Cuttlefish Tentacles
CFW	–	Cuttlefish Whole
CFWC	–	Cuttlefish Whole Cleaned
CIFT	–	Central Institute of Fisheries Technology
CMFRI	–	Central Marine Fisheries Research Institute
COD	–	Chemical Oxygen Demand
CPCB	–	Central Pollution Control Board
CPHEEO	–	Central Public Health and Environmental Engineering Organization
CST	–	Conventional Septic Tank System
DO	–	Dissolved Oxygen
DOSTE	–	Department Of Science, Technology and Environment
EIC	–	Export Inspection Council
EMB	–	Eosin Methylene Blue
ETP	–	Effluent Treatment Plant

EU	–	European Union
EVS	–	European Voluntary Service
FAO	–	Food and Agricultural Organization
FBR	–	Fluidized Bed Reactor
FDA	–	Food and Drug Administration
FFBR	–	Fixed Film Bioreactor
FREMP	–	Frazer River Estuary Management Programme
GDP	–	Gross Domestic Product
GO	–	Gutted Octopus
HACCP	–	Hazard Analysis at Critical Control Point
I CP AES	–	Inductively Coupled Plasma Atomic Emission Spectroscopy
IQF	–	Individually Quick Frozen
KLD	–	Kilo Litres of Discharge
KSPCB	–	Kerala State Pollution Control Board
LB	–	Lactose Containing Broth
MOEF	–	Ministry Of Environment and Forest
MPEDA	–	Marine Products Export Development Authority
MPN	–	Most Probable Number
NDFS	–	Nano dispersion and Flocculation System
NOB	–	Nitrite Oxidizing Bacteria
O & G	–	Oil and Grease
OLAND	–	Oxygen Limited Aerobic Nitrification – Denitrification
OWC	–	Octopus Whole Cleaned
P D	–	Peeled Deveined
PF	–	Plate Frozen
PUD	–	peeled Undeveined
QMP	–	Quality Management Practices
RBC	–	Rotating Biological Contactor
SBR	–	Sequencing Batch Reactor
SBSBR	–	Stringed Bed Suspended Bioreactor
SQRG	–	Squid Ring
SQTN	–	Squid Tentacle
SQT	–	Squid Tube

SQW	–	Squid whole
SQWC	–	Squid whole cleaned
SRT	–	Sludge Retention Time
T F	–	Tunnel Frozen
TAN	–	Total Ammonia Nitrogen
TFS	–	Trickling Filter System
TKN	–	Total Kjehldahl Nitrogen
TMA	–	Trimethyl Amine
TQM	–	Total quality Management
TS	–	Total Solids
TSS	–	Total Suspended Solids
UASB	–	Up flow Anaerobic Sludge Blanket
UNEP	–	United Nations Environment Programme
USFDA	–	United States Food and Drugs Authority

Chapter 1

INTRODUCTION

Contents

1.1 Background Information

Fisheries and aquaculture play a dominant role in making the livelihoods of millions of people around the world – from the small scale inland fishers who collect fish from lakes and swamps to the men and women who work in large processing plants. Earlier, economically and socially backward people were employed in this profession. Modern emerging technologies, mechanized fishing vessels and processing technologies have brought vast changes in the field of public fishing and seafood processing. This provide numerous jobs in ancillary activities such as processing, packaging, marketing and distribution, manufacturing of fish processing equipments, net and gear making, ice production and supply, construction and maintenance, research and administration, apart from the primary sector. Now the profession has shifted from the downtrodden communities to the hands of the industrialists and technologists. Today fishing and related activities provide employment to millions of people around the world. Nearly 10-12 percent of the world population depends on this sector.

Fish and seafood are much sought after by a broad cross-section of the world's population, particularly in developing countries, because of their nutritional and health attributes, taste and easy digestibility. Fish remains one of the most traded commodities worldwide, worth almost $130 billion in 2012, a figure which is likely to increase. In 2012, it represented about 10 percent of the total agricultural exports. Per capita fish consumption is higher than ever. It increased from 10 kg per capita in 1960 to more than 19 kg per capita in 2012 (The State of World Fisheries and Aquaculture, 2014). The projected per capita consumption of fish in the year 2020 is estimated to be 16.2 kg per capita/year and 21.5 kg per capita/year for the developing world and developed world, respectively (Delgado et al., 2003). In 2030, per capita fish consumption is estimated to be 18.2 kg (vs.9.9 kg in the 1960s and 19.2 kg in 2012). This is equivalent to another 23 million tons of seafood supply, which aquaculture will have to provide (World Bank, 2013; FAO, 2014).

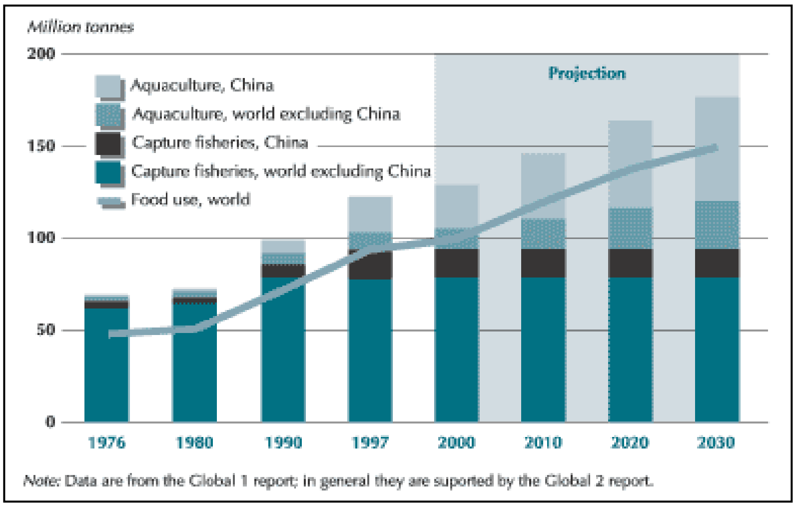
Countries with rapid population growth, rapid income growth and urbanization tend to have the greatest increase in consumption of fish. During the last two decades, a significant diversity can be observed in the utilization and processing of fish particularly into fresh and processed products, added by changing consumer tastes and development in technology, packaging, logistics and transport. This diversity includes improvement in storage and processing capacity together with innovative refrigeration facilities, ice-making, food packaging and fish processing equipments. Fishing vessels which incorporate these improved facilities are capable of staying at sea for a long time. This allows the supply of more fish in live or fresh form. Also, improved processing technology enables higher yields and more profitable products from the available raw materials.

Developed countries mainly focus on increased production of a variety of high value added products. Whereas developing countries provided with a pool of cheap labour, are still focused on less sophisticated processing methods such as, filleting, salting, canning, drying and fermenting. These labour-intensive, traditional fish processing methods provide livelihood for a large number of people living in the coastal areas. Hence these traditional methods continue to be important components in rural economies structured to promote poverty alleviation and rural development. Developing countries have boosted their share in the fishery trade by about 54 % of the fishery export by value in 2012 and more than 60 % by quantity (live weight). Fisheries and fish farming are playing an increasingly critical role for many local economies. Out of the 90% small-scale fishers, 15% are women. In the secondary activities such as fish processing, this figure can be as high as 90%.

Since 1970, the global aquaculture production has increased 40 times and is expected to quintuple in the coming 50 years (Avnimelech et al., 2008; Bosma and Verdegem, 2011). It is expected that aquaculture will contribute and strengthen food security and alleviate poverty in many developing countries. FAO (2014), estimates that fisheries and aquaculture support the livelihood of 10- 12% of the world's population. Since 1990, employment in the fisheries and aquaculture sector has grown at a faster rate than the world's population, and by 2012, it has provided jobs for about 60 million people. Of these 84% were employed in Asia followed by Africa with about 10% and by Latin America and Caribbean Islands with 4%.

Proliferation of different food industries around the world aggravated the problem of waste handling and disposal. Seafood processing industry is not different in this aspect. Seafood industries vary in terms of raw material, source of utility water and type of processing. The common process in fish processing plants are filleting, freezing, drying, fermenting, canning and smoking (Palenzuela-Rollon 1999).

Apart from the positive contribution towards economy, food security, livelihood and nutrition, this sector also contributes profoundly towards the organic waste pool of the world. The world's population is expected to increase by 36% during 2000 - 2030, from approximately 6.1 to 8.3 billion. It is also expected that the estimated total seafood demand will be 183 million tons by 2030 (Bastien, 2003). On the basis of economic models of demand, trade and supply of fish in main markets, FAO (2002) predicted the projected fish consumption and aquaculture production by 2030. The study projected China as the leading aquaculture producer in the world (Fig.1.1).



Source: www.fao.org

Fig. 1.1: World fish production and food use consumption 1976-2030

The increase in world population as well as the seafood supply and demand will lead to further elevated seafood production and processing in every continent. Ipso facto this will further aggravate the waste problem globally. Unfortunately, the quantity of wastewater generated from these processing plants, the waste load, as well as the impacts of these wastes in the environment, have not been yet properly addressed.

1.2 Seafood Industry -The Indian Scenario

India has ample potential for the development of fisheries as it is gifted with a long coastline of 8129 km, two million square kilometer of Exclusive Economic Zone and 1.2 million hectares of brackish water bodies. According to FAO, India has 8118 km of marine coastline, 3829 fishing villages and 1914 traditional fish landing centres, 1,95210 kilometres of rivers and canals, 2.9 million hectares of ponds and lakes and about 0.8 million hectares of wetlands and water bodies. 1.24 million hectares of brackish water area are suitable for shrimp farming, of which only 15 % is utilized at present for shrimp farming, producing 2.78 lakh tons of shrimp, scampi, mud crab and sea bass. In addition, about one million hectors of coastal land awaits utilization through brackish water farming and a number of protected bays and coves, along the 7517 km coastline for remunerative mariculture (Pandey et al., 2010). Against an estimated potential of 3.9 million tons from marine sector, only 2.6 million tons are tapped. Fishing efforts are largely confined to the inshore waters through artisanal, traditional and mechanized sectors (MPEDA, 2007). India's total fishery production is about 8.88 million tonnes from both capture and aquaculture. Fish output in India doubled between 1990 and 2010 (FAO 2011). India shares 2.4% of the world seafood market and ranks fourth in global fish production and second in aquaculture. 'Blue Revolution' paved the way for the growth of fish production and increasing it from 0.75 million metric tons in 1951 to 6.1 million metric tons in 2003. There is a tenfold increase in the fish production since independence in 1947. As per Central Institute of Fisheries and Technology (2008), India is a major supplier of fish in the world and in aquaculture fish production it is the second largest country. Marine and freshwater catch fishing combined with aquaculture fish farming is a rapidly growing industry in India (Handbook of fisheries and aquaculture 2013). Fisheries play an important role in the economy of India in generating employment, augmenting food supply, raising nutritional levels and earning foreign exchange. It has been regarded as a powerful source to generate income and employment and it boosts the growth of a number of subsidiary industries. According to a government release from New Delhi, fisheries sector contribute 1.10% to the total GDP and 5.3% to the agriculture GDP. FAO (2012) estimated that there was a supply gap of 25 million tons of fish globally by the end of 2000. This gap during the period 2000-2010 and 2020-2025 are expected to become about 37 MT and 63 MT respectively. India occupied the third position among the largest fish producing countries and stood 19th among the world's largest exporters of seafood with an annual export exceeding US $ 1.2 billion. It is the biggest foreign exchange earner among commodities without any important inputs and it accounts nearly 8% of the net foreign exchange earnings of the country. India exports 5.20 lakhs tons of fish fetching ₹ 8,363 crores and provides employment to 14 million people. The growth profile of Indian marine products exports during 1961-2012 is presented in Table 1.1. In India, there are 369 seafood processing units with a daily processing capacity of 10266 ton, out of which 257 units are approved by European Union (EU). There are around 1050 registered exporters among which 207 export chilled items, four export freeze dried products, 149 export dried items, 13 export canned products, 555 deals with frozen products and 191 produce other seafood and allied products (MPEDA 2013).

The main challenges faced by this industry are productivity increase and production cost reduction. The future of this industry depends on how well these requirements are being met. There will be more pressure on these industries to improve their environmental footprint and hygienic situation especially in the context of increasing export potential. Keeping in view of the global demand for Indian seafood and its contributions to the economy, quality and quantity of the imported fish should be rigorously assessed as per international standards (Annual Report 2008-2009, Anon, 1994-2002).

Table 1.1: Growth in Export of Indian Marine Products (1961- '62 to 2011 – '12)

Year	Quantity Tonnes	Value in Rs. Crore	Average Unit value Realization (Rs. / Kg)	Average Exchange Rate US $	Value in US $ Million	Average Unit Value Realization US $ / Kg.	Growth rate %		
							Quantity	Rupee Value	Dollar Value
1	2	9	4	5	6	7	8	9	10
1961-62	15732	3.92	2.49	NA	NA	NA	-21.30	-15.52	NA
1962-63	11161	4.20	3.76	NA	NA	NA	-29.06	7.14	NA
1963-64	19057	6.09	3.20	NA	NA	NA	70.75	45.00	NA
1964-65	21122	7.14	3.38	NA	NA	NA	10.84	17.24	NA
1965-66	15295	7.06	4.62	NA	NA	NA	-27.59	-1.12	NA
1966-67	21116	17.37	8.23	NA	NA	NA	38.06	146.03	NA
1967-68	21907	19.72	9.00	NA	NA	NA	3.75	13.53	NA
1968-69	26811	24.70	9.21	NA	NA	NA	22.39	25.25	NA
1969-70	31695	33.46	10.56	NA	NA	NA	18.22	35.47	NA
1970-71	35883	35.07	9.77	7.5578	46.40	1.29	13.21	4.81	NA
1971-72	35523	44.55	12.54	7.4731	59.61	1.68	- 1.00	27.03	28.47
1972-73	38903	59.72	15.35	7.6750	77.81	2.00	9.51	34.05	30.53
1973-74	52279	89.51	17.12	7.7925	114.87	2.20	34.38	49.88	47.62
1974-75	45099	68.41	15.17	7.9408	86.15	1.91	-13.73	-23.57	-25.00
1975-76	54463	124.53	22.87	8.6825	143.43	2.63	20.76	82.03	66.48
1976-77	66750	189.12	28.33	8.9775	210.66	3.16	22.56	51.87	46.88
1977-78	56967	180.12	31.62	8.5858	209.79	3.68	-14.66	-4.76	-0.41
1978-79	86894	234.62	27.00	8.2267	285.19	3.28	52.53	30.26	35.94
1979-80	86401	248.82	28.80	8.0975	307.28	3.56	-0.57	6.05	7.74
1980-81	75591	234.84	31.07	7.9092	296.92	3.93	-12.51	-5.62	-3.37
1981-82	70105	286.01	40.80	8.9683	318.91	4.55	-7.26	21.79	7.41
1982-83	78175	361.36	46.22	9.6660	373.85	4.78	11.51	26.35	17.23
1983-84	92187	373.02	40.46	10.3400	360.75	3.91	17.92	3.23	-3.50
1984-85	86187	384.29	44.59	11.8886	323.24	3.75	-6.51	3.02	-10.40

Year	Quantity Tonnes	Value in Rs. Crore	Average Unit value Realization (Rs. / Kg)	Average Exchange Rate US $	Value in US $ Million	Average Unit Value Realization US $ / Kg.	Growth rate %		
							Quantity	Rupee Value	Dollar Value
1985-86	83651	398.00	47.58	12.2349	325.30	3.89	-2.94	3.57	0.64
1986-87	85843	460.67	53.66	12.7782	360.51	4.20	2.62	15.75	10.82
1987-88	97179	531.20	54.66	12.9658	409.69	4.22	13.21	15.31	13.64
1988-89	99777	597.85	59.92	14.4817	412.83	4.14	2.67	12.55	0.77
1989-90	110843	634.99	57.29	16.6492	381.39	3.44	11.09	6.21	-7.62
1990-91	139419	893.37	64.08	17.9428	497.90	3.57	25.78	40.69	30.55
1991-92	171820	1375.89	80.08	24.4737	562.19	3.27	23.24	54.01	12.91
1992-93	209025	1768.56	84.61	28.9628	610.63	2.92	21.65	28.54	8.62
1993-94	243960	2503.62	102.62	31.3655	798.21	3.27	16.71	41.56	30.72
1994-95	307337	3575.27	116.33	31.4000	1138.62	3.70	25.98	42.80	42.65
1995-96	296277	3501.11	118.17	31.5000	1111.46	3.75	-3.60	-2.07	-2.39
1996-97	378199	4121.36	108.97	35.7500	1152.83	3.05	27.65	17.72	3.72
1997-98	385818	4697.48	121.75	36.2500	1295.86	3.36	2.01	13.98	12.41
1998-99	302934	4626.87	152.74	41.8000	1106.91	3.65	-21.48	-1.50	-14.58
1999-00	343031	5116.67	149.16	43.0300	1189.09	3.47	13.24	10.59	7.42
2000-01	440473	6443.89	146.29	45.4975	1416.32	3.22	28.41	25.94	19.11
2001-02	424470	5957.05	140.34	47.5292	1253.35	2.95	-3.63	-7.56	-11.51
2002-03	467297	6881.31	147.26	48.2933	1424.90	3.05	10.09	15.52	13.69
2003-04	412017	6091.95	147.86	45.7091	1330.76	3.23	-11.83	-11.47	-6.61
2004-05	461329	6646.69	144.08	44.6683	1478.48	3.20	11.97	9.11	11.10
2005-06	512164	7245.30	141.46	44.0655	1644.21	3.21	11.02	9.05	11.21
2006-07	612641	8363.53	136.52	45.1367	1852.93	3.02	19.62	15.43	12.69
2007-08	541701	7620.92	140.68	40.1293	1899.09	3.51	-11.58	-8.88	2.49
2008-09	602835	8607.94	145.79	45.99	1908.63	3.17	11.29	12.95	00.50
2009-10	678436	10048.53	148.11	47.11	2132.84	3.14	12.54	16.74	11.75
2010-11	813091	12901.47	158.67	45.5548	2856.92	3.51	19.85	28.39	33.95
2011-12	862021	16597.23	192.54	47.31	3508.45	4.07	26.02	28.65	22.81

Source : MPEDA, 2013

The Indian seafood industry today is totally different as to what existed in the past. India achieved a major position in the Global Seafood Market and our seafood is one of the biggest foreign exchange earners. There are world-class seafood processing and exporting factories which follow quality control procedures according to the most stringent international standards. The industry has diversified its product range and its markets, even though shrimp continues to dominate our export basket. Japan was India's largest market for many years, but recently United States has emerged as our leading export market. Our exports to US are value added products, whereas to Japan it is basic raw materials for reprocessing.

The four biggest seafood exporting ports in India are Chennai, Mumbai, Kochi and Visakhapattanam, with 30, 13, 10 and 8% share by value and 11, 23, 12 and 21% by volume respectively. During the financial year 2014-'15 marine products exports reached an all-time high record of US $ 5511.12 million. It crossed all the previous records in quantity, rupee value and US $ terms. Export aggregated to 1051243 MT valued at ₹ 33441.61 crore and US $ 5511.12 million. Compared with the previous year (2013-'14), seafood exports recorded a growth of 6.86% in quantity, 10.69% in rupee and 10.05 % growth in US $ earnings (Table 1.2).

Table 1.2: Export Performance During 2014-15 compared to 2013-14

Export Details	2014-15	2013-14	Growth %
Quantity in Tonnes	1051243	983756	6.86
Value in 10 lakhs	334416.06	302132.60	10.69
USD in '0000	551111.85	500769.75	10.05
Unit Value (USD/Kg)	5.24	5.09	2.99

Source: Press Information Bureau (2015).

The increasing trend in aquaculture has been significantly contributing to seafood exports. Emerging technology, such as pathogen free shrimp species *Litopenaeus vannamei*, is playing a major role in seafood exports, seafood shipments had increased by about 4,000 tonnes. Since 2013, frozen shrimps (vannamei shrimp) had become the principal export item in marine products.

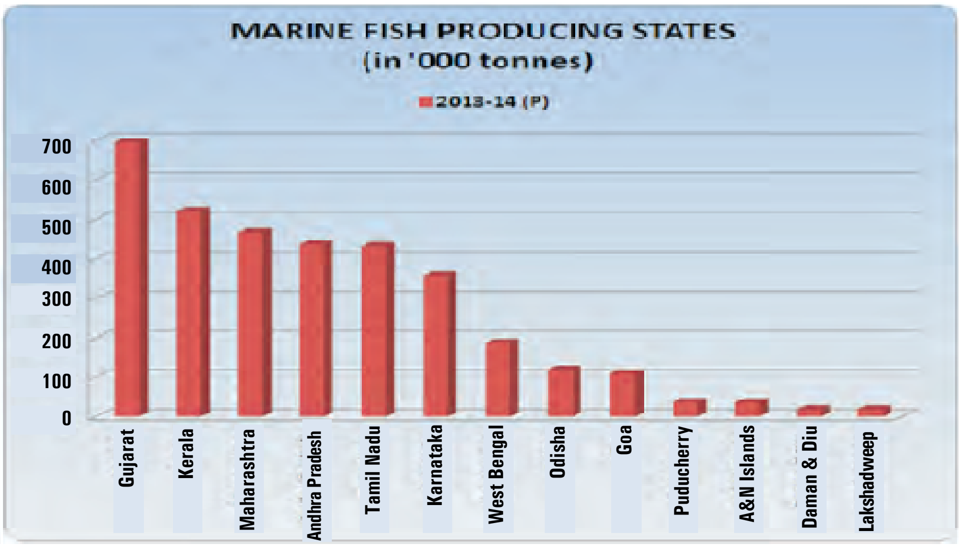
The world's biggest importer of fish, seafood and aquaculture products is the European Union. The import rules for these products apply in all EU countries as they are harmonized. The European Commission is the negotiating partner that defines import conditions and certification requirements for non-EU countries. General for Health and Consumers (SANCO), the European Commission's Directorate is responsible for food safety in EU.

As the importing countries have stringent food safety rules, consequently Indian seafood industry has been forced to upgrade its infrastructure and processing technologies to meet these standards and to comply with various quality standards like Codes standards, United States Food and Drugs Authority (USFDA) standards, European Union (EU) norms, Bureau of Indian Standards (BIS) etc. at the national as well as International levels. To meet these standards there are several quality assurance programmes developed and practised. The HACCP (Hazard Analysis at Critical Control Point) system of USA, the European Council Directives, the QMP (Quality Management Practices) of Canada and TQM (Total Quality Management) of Japan are aimed to ensure quality of the fishery products consumed in those countries. Despite, these quality assurance laws and acts assured conformance to the quality of the products as per International standards, it is not practiced in maintaining the quality of the environment into which the processing wastes are discharged. Seafood processing in any form generates excessive quantities of waste which have a negative impact on the environment as well as to the inhabitants. Due to its high organic content, seafood waste is often classified as a certified waste which is costly to dispose (Knuckey, et al., 2004).

In India there are many laws to safeguard the environment armed with strict instructions on solid and liquid waste management such as Environmental Protection Act 1986, Water (Prevention and Control) Act, 1974 and the Municipal Solid Waste (Management and Handling) rules, 2000. In spite of this, waste management scenario in India, particularly of organic waste has been facing a serious setback.

1.3 Kerala Scenario

Kerala, one of the southernmost littoral states of India stretching about 590 km near the Arabian Sea, contribute significantly to the Indian seafood industry with 287 seafood exporters, including 124 processing plants of which 62 are approved by European Union, 169 cold storages, for which the raw materials are supplied by a fleet of 4000 mechanized vessels, 16,000 small motor boats and 3,000 traditional crafts (Table 1.3). Kerala holds a top position among the maritime states in India, in marine fish production (Fig.1.3).

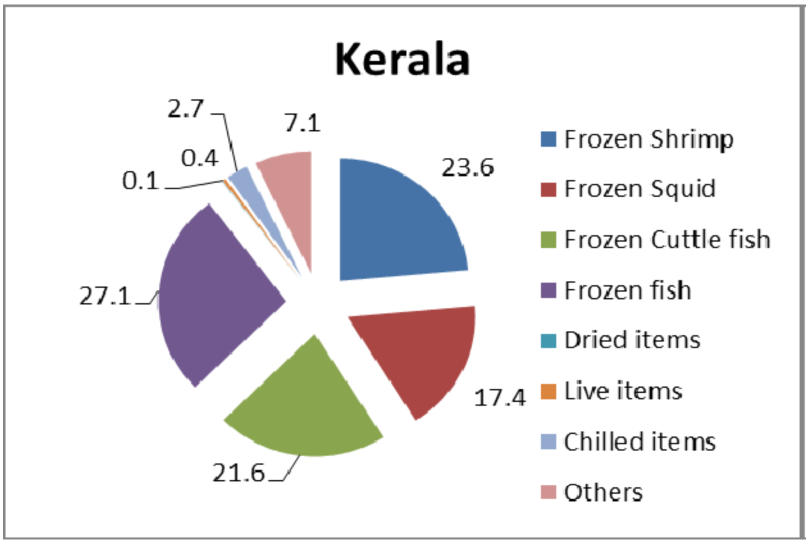


Source: Handbook on Fisheries Statistics 2014

Fig. 1.2: Marine fish producing states

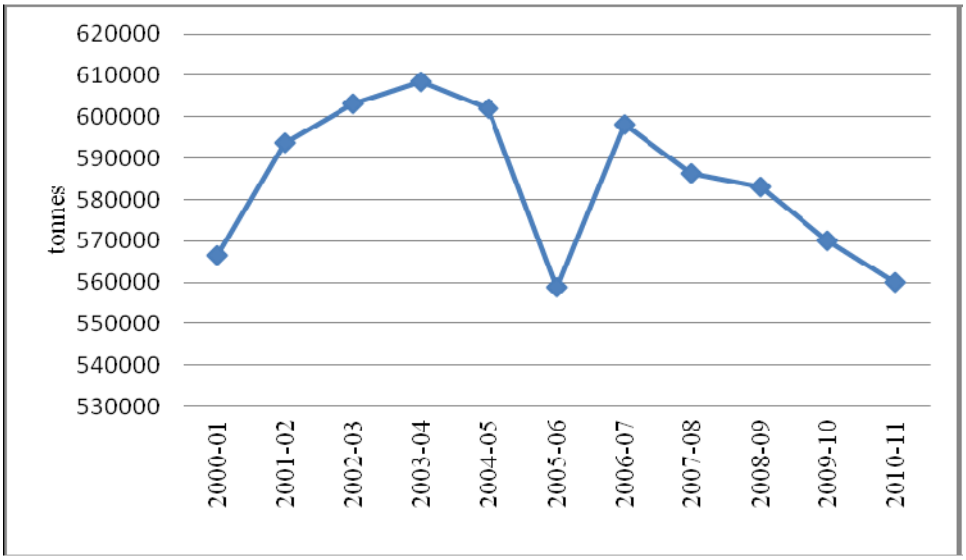
The share of total marine fish production in Kerala is higher than that of the National average. Mud Banks locally called "Chakara" a unique phenomenon which occurs in Kerala coast during Monsoon months contributes significantly towards a good harvest of fish. The marine fishery dominates the total fish production compared to inland fisheries in the state attaining the optimum level of production, though the inland fishery is showing an increasing trend. Inland fish production has also increased since late 1980s due to the rise of aquaculture in the state. The inland fish production reached a peak share of 13% of the total fish production of the state during 2000-'01 and thereafter declined slightly (Economic Review, 2007). Out of the total 5.83 lakh tonnes of fish production in 2008-'09, 1.01 lakh tonnes were exported fetching ₹ 1570 crore. Kochi is the major port through which a lion share of seafood leaves off to export markets, which has resulted in a large number of seafood and allied industries concentrating in and around this area together with the Aroor panchayath of Alappuzha district jointly becoming a major seafood processing hub in Kerala, and in India as a whole, by the presence of the highest concentration of processing and pre-processing units in the country.

Compared to 2011 the total marine fish landing along the Kerala coast was more in 2012, an increase of 96,062 t (12.9%) was observed. This difference is due to the increased production of pelagic resources. The major items contributed to this were oil sardine (47.6%), Indian mackerel (4.8%), stoleophorus (4.6%), penaeid prawns (4.9%), threadfin breams (7.1%), carangids (7.0%), cephalopods (4.9%), flat fishes (2.6%), tunas (2.0%), ribbon fish (1.5%) etc. Major crustacean resources like penaeid prawns, crabs and stomatopods also have increased (CMFRI Annual Report 2012-2013). In terms of value, the main seafood varieties that are exported from Kerala are shrimp, cuttlefish, squid and fin fishes. Figure 1.3 details Kerala's share in the Indian export market in 2012 where frozen fish was the major export item followed by frozen shrimp, cuttlefish and squid (Directorate of fisheries, 2012). The four main shrimp varieties which are in export market are Naran (*Peneaus indicus*), poovalan, karikadi (*Parapenaeopsis stylifera*) and karachemmen (*Peneaus monodon*) obtained from capture and culture sources. Important among fin fishes in the export basket are reef cods, emperor breams, white snapper and seer fish.



Source: Director of Fisheries. 2012.

Fig. 1.3: Kerala's share in the Indian Export Market



Source: Handbook on Fisheries Statistics 2011.

Fig. 1.4: Trends in Marine Fishing in Kerala: 2000–01 to 2010–11 (tonnes)

Kerala seafood export industry is facing various problems; first among them is the raw material scarcity (Fig.1.4). Fluctuation in the catch quantities coupled with overcapacity in the fishing fleet is placing enormous pressure on the profitability of individual fishing units. This problem is further aggravated by the steady increase in the operating cost. The peeling shed industry and processing industry in the state is also affected by the raw material scarcity. When the demand for processed seafood continues to exist in the export markets, the non-availability of sufficient quantities of raw material at a reasonable price keeps many processing plants idle. The additional processing capacity that has been built up at a high cost is now proving to be a handicap for many processors.

The pre-processing plants, locally called 'peeling sheds', play a vital role in the fishery sector of Kerala. It is mandatory to have a full-fledged pre-processing unit integrated with the processing unit. In other parts of India pre-processing of crustaceans and cephalopods, including de-shelling and cleaning, is integrated with the processing facilities, whereas in Kerala, most tend to outsource it in order to cope with the labour as well as fluctuating raw material availability and its cost. It is undertaken by independent pre-processing facilities, the so-called 'peeling sheds', which operate with lower capital and are less organized. Women dominate the peeling sheds where shrimp, squid, cuttlefish etc. are brought to peel. They collect the raw materials from various landing centres including those outside the state, which are then peeled, cleaned, graded and sold to the processing factories for further processing and exporting. They are playing a major role in the seafood industry as they reduce the raw material collection cost and pre processing cost of export processors. Pre processing is an extremely labour intensive operation depending on the supply of skilled labourers flexible enough to cope with significant variations of raw material supply from day to day and season to season.

House peeling practices were very common in Kerala until the late 1980 s, even though it accounted for a relatively small percentage of the total output. Women, especially Muslims who are homemakers, in their leisure time take part in house peeling and thereby earn some money. They collect the raw material and peel in their own home. This practice invited inspection by Export Inspection Council (EIC) and Marine Products Export Development Authority (MPEDA). They voted for the eradication of the home peeling practice thereby leading to the merging of pre-processing units to the processing plants.

EIC has approved about 125 pre-processing units, all of which are linked to the processing facilities under their direct control. At the same time the number of pre-processing plants declined to 58% from 1997-98 to 2003-04, whereas the installed capacity increased by 42% from 2,700 tonnes/ day to 3,860 tonnes/day during that period.

Even then independent peeling sheds which worked on a seasonal basis and house peeling practices are prevalent in some areas where there is abundant supply of raw material in some seasons. Most of these peeling sheds are located in and around Kochi especially in Aroor - Chandiroor region. A large number of small processing facilities also support the processing, particularly of shrimp in Kerala.

One of the most important negative externalities generated by seafood industry is the pollution by waste. In fact all industries produce waste as a result of manufacturing process. Unlike, chemical wastes which has a profound impact on the environment whenever it is released above the permissible limits, food processing wastes usually escape unnoticed, until its impact on the environment has reached to an uncontrollable proportion.

The peeling sheds, house peeling practices and the small processors generate a large amount of waste on a seasonal basis and discharge this untreated or partly treated wastewater to the nearby canals or backwaters which adversely affect the coastal waters and local drinking water sources. The pollution control board doubtlessly demands more advanced waste water treatment methods. Therefore an eco friendly and economically acceptable practical treatment option has to be implemented in all these facilities.

Table 1.3: Fisheries profile of Kerala

Coastline of Kerala	590 km
Marine fishing villages	222
Inland fishing villages	113
Fishing harbours	10
Fish landing centres	61
Whole sale fish markets	185
Retail fish market	2518
EU approved processing plants	62
Prawn filtration fields	6,129 hector
Public sector brackish water farms	2,873 hector
Fishing crafts	21746
Marine fishermen population	879800
Marine fish production	570013 MT
Inland fish production	116836 MT
Export through the ports of Kerala	
Quantity in M.Tons	107293
Value in Rs. Cr.	1670
Percentage share in terms of Quantity in export	16
Percentage share of Kerala in terms of Value in export	17

Source: KMFS, 2010, Directorate of Economics and Statistics, Govt. of Kerala

1.4 Problem of the study

Major challenges faced by the people of littoral communities especially in developing countries like India, is the negative externalities exerted by the seafood industries on their environment that negatively affects their livelihood. Though these industries have created certain positive impacts like job opportunities and infrastructure facilities, it is the negative effects that have created an in-depth impact on the local communities. Seafood processing industry primarily depends on many factors like raw material availability, its cost, and products' market and so on. In addition to that, like many other foods processing industries seafood processing facilities have many environmental impacts such as high water consumption, energy consumption and discharge of effluents with high organic load. *Though there are effluent treatment plants in the processing facilities, they are not often operated by the owners because of the high electricity charges, labour and maintenance charge and the organic effluents immensely affect the aquatic ecosystem where it is drained to and in turn the ground water.*

1.5 Significance of the Study Area

Aroor the gateway to the 'Venice of the East' Alappuzha District in Kerala alone contributes 60% of India's seafood industry. An abundance of processing and exporting and pre processing units are located throughout this locality. Most of these industries are located on the fringes of Vembanadu lake and its tributary canals. Four panchayaths, Aroor, Ezhupunna, Koodamthuruth and Kuthiathodu in Cherthala thaluk of Alappuzha district were declared first in the country as "The Town of

Export Excellence" in the marine sector (The Financial Express, Feb 27 2005). There are 47 processing plants, two large cold storages, 130 ice plants, 175 pre processing centres and several unregistered house peeling facilities. Regarding water supply, it is estimated that in this area the daily consumption, mostly groundwater by these processing sector is around 35 lakh litres, of which a good part is being used for washing and then drained out often without adequate treatment, which ultimately reaches the water body.

Fig. 1.5: Map showing the Special Economic Zone, Aroor seafood industry zone

This wastewater contributes significantly to the pollution load of the water bodies leading to serious ecological ramifications. The hydrological (ultimately environmental) implication of this unregulated water extraction is not yet studied properly. The boon of this area, which provides employment mostly to the underprivileged and earning invaluable foreign exchange to the country, is now a bane to the local residents suffering from serious health ramifications.

It is an area of environmental concern which is continuously making news for being an area of severe environmental crisis affecting life of inhabitants. Irrespective of the interim corrective actions taken by the regulatory authorities, the issue of pollution seems to persist for a long period of time. Media and the authorities have been accusing this industry for irresponsibly discharging untreated or improperly treated effluents to the adjacent water bodies. In addition, two matters of serious concern are the tremendous increase in the vector population as well as the rendering of the canals and backwaters as almost dead pools, resulting in dramatic decrease in the fishery resources. People of this locality depend on this interconnected water canals for water and other livelihood requirements also aggravate the effects of pollution resulting in serious social resistance. The canals of this area are connected with Vembanadu Lake, one of the ecologically significant water bodies in India which makes the issue more pivotal. Vembanadu lake is one of the major estuaries in India situated between latitudes $9^{o}28'$ and $10^{o}10'$N and longitudes $76^{o}13'$ and $76^{o}31'$ E in southern Kerala with a length of 90 km extending from Alappuzha to Azheekodu with a water spread area of 300sq.km. There are many studies indicating the role of seafood processing companies in a variety of pollution issues in this area. These studies largely concentrate on the receiving end, projecting the changes in physio-chemical parameters of the polluted water bodies.

1.6 Area of special interest

Chandiroor canal (Puthenthodu), located in Aroor panchayat which extends to a length of 1.4 km and width of 12.5 meters connected on both ends by backwaters. Pollution in the canal is very high due to the discharge of effluents from 11 seafood processing plants and 5 peeling sheds (Fig.1.6 a & b). The pollution of this canal from seafood effluent discharge is persisting for more than 25 years and has been exposed to violent agitations with no or little effect. A report of the State Pollution Control Board in 2002-03 had construed Aroor in Cherthala Thaluk as the most polluted Panchayat (Village) in the state. In addition, Chandiroor canal flowing through this area had been labelled as the most contaminated canal of the state (Devraj, 2006)

A survey conducted (Santhigiri Siddha Medical College, Medical Survey, 2006) by the medical college revealed that the inhabitants of this area were suffering from rheumatic complaints, respiratory ailments like asthma, bronchitis, skin diseases, diabetes mellitus, hypertension, piles etc.. Reason for the above-said disorders were probably water contamination from seafood industries and its allied sector, ice plants. Water stagnation made it a breeding ground for mosquitoes, flies and other vectors making people's life literally a hell.

A common effluent treatment plant was Chandiroors' dream. Yester years provided, this dream come true for the residents, fund was allowed for the project, but the non availability of best suited area pushed the authority's attempts futile and the fund was lapsed. However, recently, January 2016, land was acquired and the fund was re-allotted as per the loud cry of residents, local body authorities and the processors. The local people face many problems including the following:-

- Obnoxious odours and breathing problems
- Lack of pure water to drink
- Ground water pollution
- Skin diseases and gastric problems
- Uncontrolled vector population like mosquitoes and houseflies
- Fish mortality
- Crop destruction by wastewater infiltration
- Chocking and clogging of water canal.

1.7 Precise Objectives

- To understand, the present processing technologies, waste generation and treatment facilities.
- Characterization of the liquid waste generated in the fish pre-processing and processing centres.
- Assessment of the efficacy of the existing treatment systems.
- Testing of Stringed Bed Suspended Bio Reactor (SBSBR) to suit the requirements of the processing centres.

1.8 Methodology

- By conducting field studies and survey in different seafood processing industries and zones to assess the present processing technologies, waste generation and treatment facilities.
- Quantification of water requirements and discharge volumes will be done by conducting on site studies.
- Effluent water quality assessments.
- Experimenting with an appropriate treatment system- Stringed Bed Suspended Bioreactor (SBSBR) augmented with an organic matter degrading bacterium.

1.9 Significance of the study

Seafood processing and exporting is the backbone of Indian economy, facing a major challenge to increase productivity and reduce production cost. There is more pressure on these industries to improve their environmental and hygienic situation. Generally effluent treatment is highly complex and uneconomical, with increasing pollution complexity and high power consumption. In fact, industry is faced with increasing problems of waste handling and disposal, plant sanitation, raw material availability and its cost, increasing labour and energy cost, and if the waste water treatment and operating cost increase alarmingly, many plants would not find it profitable to stay in business.

Each research activity is aimed to generate some significant results. The outcome of this study may give a comprehensive knowledge about the present national and local scenario of seafood industry, and the idea of treating seafood effluent by using SBSBR would be a stepping stone to develop an economically viable treatment method which is adaptable to the seasonal fluctuations.

Fig.1.6 (a): Different locations of Seafood effluent outlets to Chandiroor canal

Fig 1.6 (b): Different locations of Seafood effluent outlets to Chandiroor Canal

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Chapter **2**

ASSESSMENT OF THE PRESENT PROCESSING TECHNOLOGIES, WASTE GENERATION AND TREATMENT FACILITIES

Contents

2.1 Introduction

In the global fishery production, on an average, 30-40% is consumed as fresh and the rest is processed for human consumption and other purposes. Over the last decade the proportion of the total fishery production that is processed remained almost stable. At the same time the total bulk of the fishery commodity increased due to the steady increase in the total fishery production.

In recent years, Indian seafood processing industry gained a renowned name due to the increase in demand of processed food by the developed countries as ready to eat form. The main reason for this huge demand for the value added products are their increased economic security, better purchasing power, better education and awareness about health and hygiene. The influential factors that decide the market price and acceptability of the products are the prudent sanitation, hygiene and quality assurance of the processed products.

In India, the marine fish landing consists of about 65 commercially important species/ groups. Shrimp is the most important variety because of its export potential, even though it contributes only 10% of the total marine fish landings. Other commercial items are Indian oil sardine (*Sardinella longiceps*), Indian mackerel *(Rastrelliger kanagurta*) and anchovies constituting the main bulk of pelagic species caught followed by Bombay duck (*Harpodon nephorrius*), Seer fish (*Scomberomorus sp*.), Tunnies and Cephalopods, Sciaenids, carangids, perches and Elasmobranches.

The process used in the seafood industry generally include the following; harvesting, storing, receiving, peeling, eviscerating, picking or cleaning, preserving and packing. Harvesting may be considered as a separate industry which supplies the basic raw material for processing and further distribution to the consumer. The receiving operation usually involves three steps, vessel unloading, weighing, and transporting by a suitable container to the processing area. The catch may be processed immediately or transferred to the cold storage before processing.

Pre-processing refers to the initial steps taken before the raw material enters the plant. It may include peeling, deheading, deskinning, eviscerating fish or shellfish, and other operations to prepare the fish for processing. For squid, the pre-processing activity involves separating the dark, spotty skin of the squid from the flesh, removal of beak, cuttle bone and thoroughly washing the inside of the squid body tube. The procedures like freezing, processes for value addition like canning, filleting, breading etc. are included in the processing, intended to increase the shelf life. If the product is to be held for a long time before consumption, there are several forms of preservation methods to prevent spoilage caused by bacterial action and autolysis: such as freezing, canning and refrigeration. As bacterial growth can be arrested below -9^{o}C, freezing is one of the best methods of holding uncooked fish for a long period of time.

Wastes from the pre-processing and processing industry are screened from the waste stream and processed as a fishery by-product.

2.2 Process Description

A preliminary study was conducted by onsite visits to understand the technological aspects such as the type of raw materials used, processing methods and the wastewater treatment facilities used in the study area.

2.2.1 Major Types of Seafood Processing

2.2.1.1 Shrimp processing

The whole shrimp is iced and packed to the processing facility, where the raw shrimp are kept in ice for about two days after catching to allow proteolytic enzymes and micro organisms to break down connective tissue between meat and shell to improve peelability. This deterioration also increases water-holding capacity and the holding-period results in an increased bacterial load on the raw shrimp (Nielsen et al., 1983). Iced shrimps are dumped into melt tank for thawing, and then it is distributed to the pre-processing table for peeling (Fig. 2.1). There is a continuous flow of tap water, which loosens and washes away waste, peeling as well as washing at the same time. According to the buyer's requirement the meat is either PUD (peeled un-deveined) or PD (peeled and deveined) and sorted according to different counts. Waste and the sprayed water are flumed away to a waste sump and the effluent is drained through the floor drains. The peeled shrimp/meat is supplied to the processing section where it is checked for quality, ie, freshness, chemical residues etc. It is accepted only if it meets the minimum standards. The product is then washed in washing machine or bubbling machine. From the bubbling tank the washed meat is passed through the draining table, checked again for impurities, sorted and graded and it is block frozen (BF) or individually quick frozen (IQF) depending on the market demand or order. The material is then packed and transferred to freezers, where it is stored at -18^{o}C until it is exported. The flume water is drained through the draining table onto the floor, from where it is discharged through the mesh to the drain.

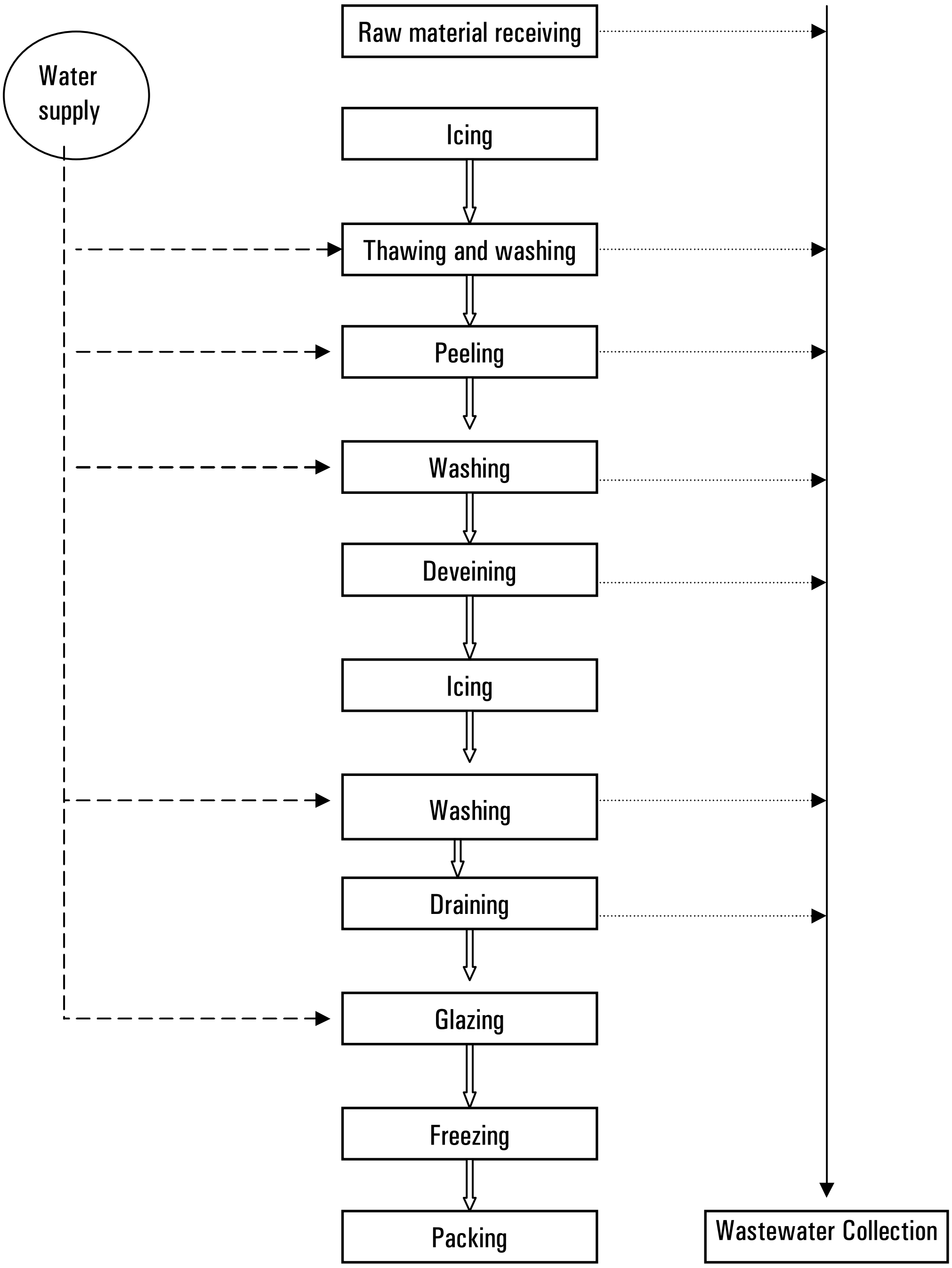

Fig. 2.1: Flow Diagram which shows Shrimp Processing

2.2.2 Cephalopod Processing

Cephalopods - mainly cuttlefish, squid and octopus are abundant in the month of July, August, September, October and November. Raw material is received in bulk quantities depending up on the plant capacity where it is washed, weighed and iced overnight. Goods are thawed in large tubs by adding water and supplied by square sieves to the peeling table, where the skin, viscera, cuttle bone and ink sack are removed under running tap water. Ink, viscera and cuttle bone are collected for by-product recovery. The wastewater is drained from the peeling table to the wastewater streams. 8 ppm Hydrogen Peroxide in water and concentrated sodium chloride solution (for 100 kg meat 80 litres of conc. salt solution to 120 litres of water with 24 ppm of Hydrogen peroxide) is added so that they may absorb water and gain weight. This treatment also makes the meat whiter and hence more acceptable to the processing plants. From this solution the meat is taken by the strainer to the cleaning table for further cleaning to remove the remaining stain or dirt, after which the meat is sorted, iced and sent to the processing plant.

Once it reaches the processing plant, it is checked for freshness, chemical residues etc. and the material is accepted only if it meets minimum standards. The meat is washed and is taken to the draining table to drain all water from the tube, set to freeze as block frozen (BF), or individually quick frozen (IQF) according to the market demands. The material is then packed and transferred to freezers where it is stored at -18^0 C until it is exported.

2.2.2.1 Types of Cephalopod Processing

SQW-Squid Whole, SQWC-Squid whole cleaned, SQRG-Squid Ring,

SQTN-Squid tentacles, SQT-Squid tube.

CFW-Cuttlefish whole, CFWC-Cuttlefish whole cleaned,

CFTN-Cuttlefish tentacles, CFFT-Cuttlefish filleting.

GO - Gutted octopus, OWC - Octopus whole cleaned.

IQF- Individual quick freezing-The meat is agitated in an agitator where there is hydrogen peroxide, sodium chloride and ice. Then the agitated meat undergoes another process called blanching. Here the agitated goods are dipped in boiled water for about 30 seconds and then in chilled water. Then it is passed through the Individual quick freezer and the product is ready for packing.

2.2.2.2 Cuttlefish filleting

The cleaned cuttlefish undergoes a trimming procedure, ie, cutting the wings and tentacles and the remaining sheet of body is packed as fillets.

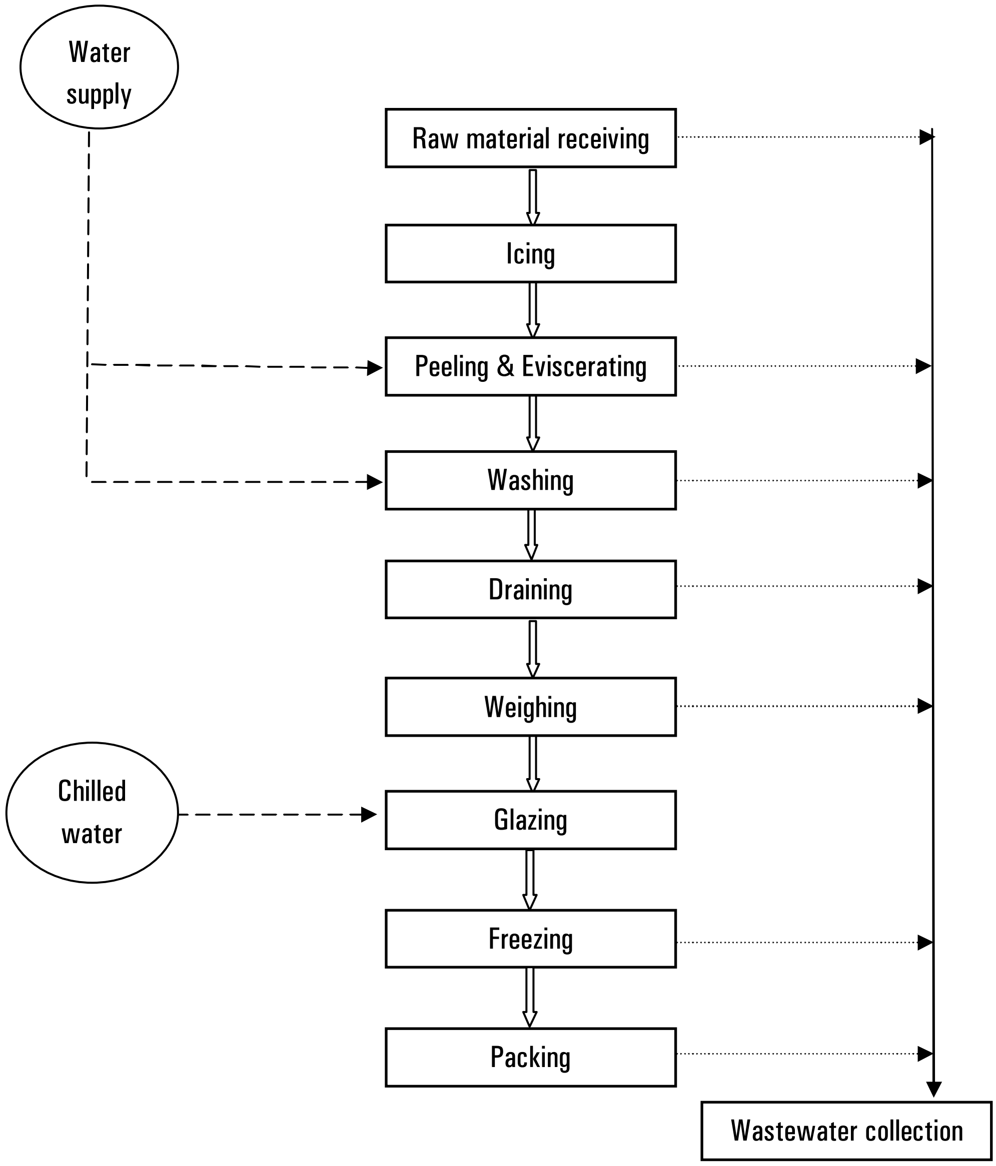

Fig. 2.2: Flow Diagram that shows Cephalopod Processing

2.2.3 Fish Processing

2.2.3.1 Fish whole packing

This is the fourth most important item in the export basket accounting for 28% by volume and 9% in value terms. Many varieties of finfishes are exported from Kerala, of which the main among them are sardine, mackerel, ribbon fish, yellow fin tuna, skipjack, pomfret (white and black) pearl spot, seer fish, reefcord, emperor breams, white snapper etc.

Yellow fin tuna, skipjack, reefcord and king fish are packed as whole and gutted or headless and gutted. All the ground fish species are pre-processed mostly in the same manner (Riddle and Shikaze1973). Virtually none of the pre-processing activity for finfishes is carried out in the peeling shed like in the case of shrimp, cuttlefish or squid. It is mainly because in comparison they do not require much pre-processing. Hence it is carried out in the pre-processing units attached to processing plants. Even though the pre-processing of fin fishes is not labour intensive, it requires high degree of skill as the yield depends on a large extent on this factor. Dressing fish for freezing involves the removal of the head and gutting of the fish. The tail, fins and the collar bone immediately behind the head are not cut off. The eggs of the female fish are removed for further processing and the milt of the male is removed at this stage. The dressing line consists of a large table and a fish cleaning station, where workers are responsible for specific tasks, such as head removal; belly slitting, removal of viscera and separation of milt and /or roe, removal of kidney and cleaning of fish. The final cleaning of the fish is done with a spoon which is directly attached to a water hose to both scrape and flush remaining viscera and blood away. Offal from dressing tables may be dropped on the floor, into net baskets for collection. The dressed fish are then washed, graded and frozen.

2.2.3.2 Fish Filleting

Most ground fish require no pre-treatment before filleting (Riddle and Shikazae, 1973), the fish are first washed in large wash tanks or by water sprays. Next the fish is passed to the filleting tables and the skin is removed, the skinned fillets are transported to a wash tank or dip tank (Fig. 2.3). After inspection the fillets are packed. Excessively high strength effluent is commonly produced as a result of trimmings either being flicked into the central tub, where they are left soaking for long periods, or being flicked directly onto the floor. In some companies a large amount of waste also ends up on the floor as a result of careless throwing towards the fish meal bin. Baskets are positioned under the drain hole in the tub to catch the trimmings; effluent strength is increased as the effluent washes through the waste.

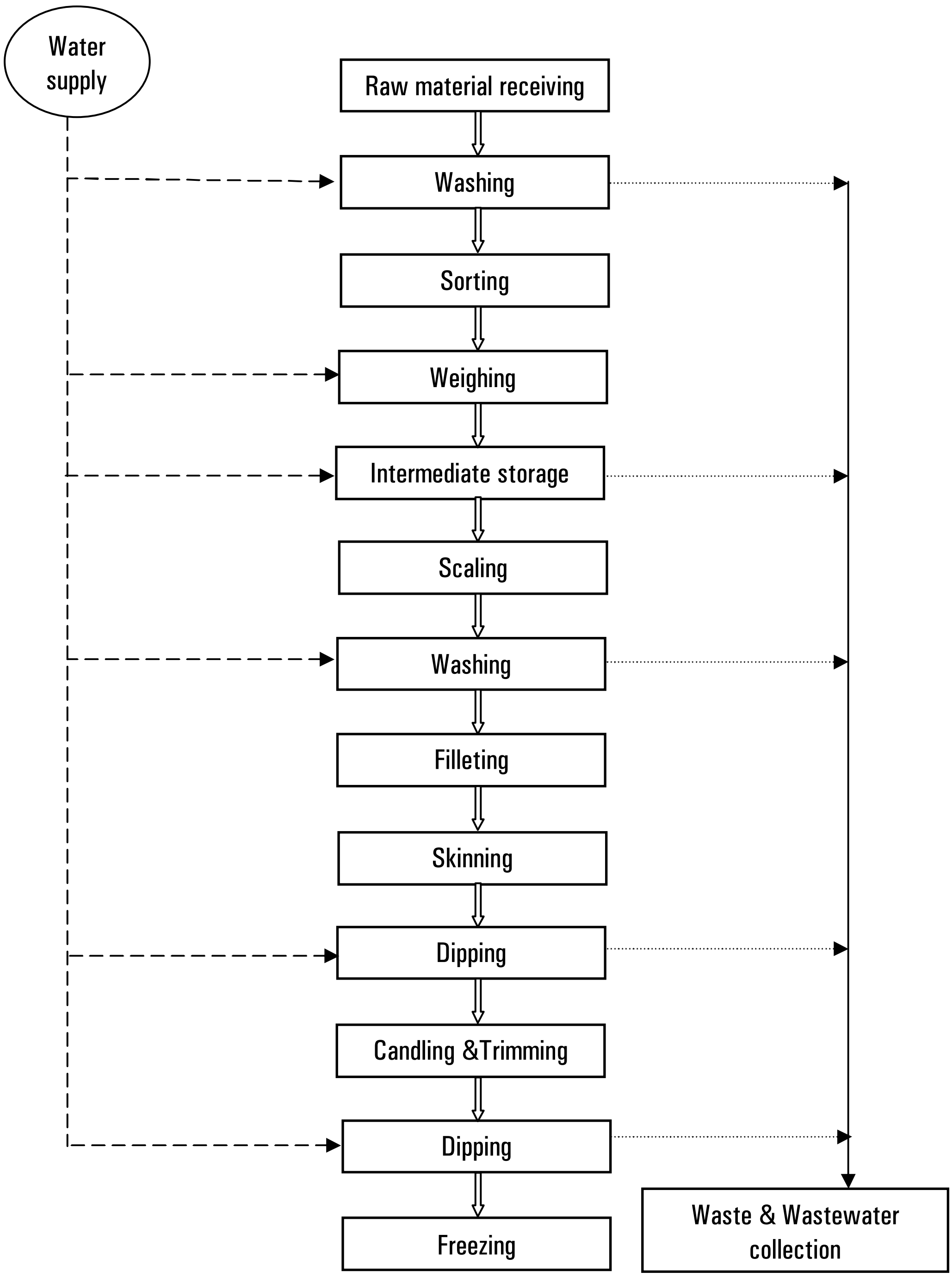

Fig. 2.3: Flow Diagram depicts fish filleting process

The above noted processing schemes have been presented to identify the various types of operations specific for the seafood industry. The industries selected were chosen primarily to exhibit the range of process activities in the seafood industry. The next chapter will identify waste characteristics related to some of the more significant operations.

2.3 Materials and Methods

2.3.1 Survey

An exclusive survey was conducted during the period between August to October 2012 in the selected pre-processing and processing plants of Aroor Ezhupunna, Kodamthuruth and Kuthiathodu Gramapanchayath and collected a one year data. The specific objective of the survey was to understand the processing technology, to quantify the volume of wastewater generated and its characteristics and the processes adopted for the treatment of wastewater. For the qualitative analysis samples were collected from some selected facilities and analyzed in the laboratory. The survey was intended for the quantitative analysis.

2.3.1.1 Basis of questionnaire development

Proper questionnaire development is guided by the application of several basic principles as well as knowing what types of questions to ask in order to effectively assess the desired attributes as it was an exploratory research. The questions were close ended that required the participants to choose from a limited number of responses and provide quantitative data (Appendix, p. 217).

The questionnaire was taken to the preprocessing and processing plants to collect the data. Each question was asked individually to ensure correctness and active participation from each facilities. A total of 10 pre-processing and 20 processing facilities were visited to collect the following data (Table 2.1 & 2.2).

- Raw materials: The magnitude of pollution by a processing plant is mainly dependant on the quantity of raw materials processed. Data regarding type, quantity, source, availability of raw materials, were collected and an evaluation of the installed capacity and utilized capacity was made.
- End products: Type of end products and its quantity
- Wastewater: Quantity, disposal mechanism, its efficiency and economic feasibility, quality of discharge and issues.

This survey gave the details of the production capacity, types and quantities of raw materials processed, type of end products, water consumption, quantity of effluent generated, energy cost, methods of wastewater treatment and maintenance cost.

2.4 Results and Discussion

2.4.1 Plant location

The factors that determines the environmental effect of the waste generation is mainly based on the nature of the site, proximity to a water body, where the facilities dispose its waste. All the processing plants surveyed are functional within a range of 200 to 500m distance from a water body such as canal, pond or backwaters (kayal). Majority of them are located near backwaters (Kayal). Almost all the peeling shed surveyed are located very close to the backwaters.

2.4.2 Source of Raw Materials

Major landing centers in Kerala are Vizhinjam, Neendakara, Sakthikulangara, Kochi, Munambam and Beypore. From these landing centres the processing units collect the raw materials according to availability and market demands. Depending upon the seasonal fluctuation and availability, they collect the raw materials from fish landing centers of neighbouring states, such as Vizhakapattanam, Mangalore, Nagapattanam, or Kanyakumari. Also, there are many aquaculture farms which supply raw materials to these industries.

2.4.3 Type of Raw Materials used

Raw materials processed were different in different processing plants surveyed, depending upon the seasonal availability of raw materials. The species processed are Shrimp (Vannaamei, Choodan, Karikkady, Thelly, Tiger prawn), cephalopods (Cuttlefish, squid and octopus) and saltwater fishes (Yellowfin Tuna, Anchovy, Ribbon fish, Mackerel, sardine and Pomfret). Penaeid shrimp is the major raw material processed in the seafood industries. Though Kochi port is second in quantity-wise export of marine products from India, price-wise it ranks first (MPEDA Annual Report, 2007-08). This is because of the large quantity of penaeid shrimp exported from Kochi, which bring more value. Out of the 20 processing plants surveyed 16 units process all species depending upon the availability, 2 units process only shrimp, 1 plant process only fin fishes and 1 plant use cephalopods as the raw material. Since frozen shrimp is the major export item, iced and peeled shrimps are the major raw material for the production processes in processing plants.

European Union give strict regulations to the processing plants that the peeling works should be done in the premises of the respective processing plant itself to maintain better hygienic practices. But only 3 of the processing plants surveyed follow this regulation and the remaining collect peeled and iced raw materials from their own peeling sheds or from outside sources.

2.4.4 Nature of Processing and Final Products

The quantum of pollution from a processing plant is highly depend on the type and quantity of raw materials processed. There are no marked diffference between the processing centres with regard to physical facilities, type of raw materials used and the type of end products (Table 2.1). Nature of processing mainly depends upon the raw material availability and the number of freezers in the plants. End products are decided by the processors in response to the market demand. The facilities available in various plants are:

i) Individual Quick Freezing

ii) Air Blast Freezing

iii) Contact Plate Freezing and

iv) Tunnel Freezing.

The nature of processing also varies in plants according to the market conditions. The major products are IQF shrimps, de-headed, headed, peeled-undeveined (PUD), peeled–deveined (PD), IQF fish, headed and gutted, headless and gutted, fresh filleting, squid whole(SQW), squid whole cleaned (SQWC), squid ring (SQRG), squid tentacles(SQTN) and squid tube (SQT), cuttlefish filleting (CFFT), cuttlefish whole (CFW), cuttlefish whole cleaned (CFWC) and cuttlefish tentacles (CFTN), gutted octopus (GO), octopus whole cleaned (OWC) (Table 2.1). Regarding peeling sheds, there is no noticeable difference between the type of raw material used or the end product (Table 2.2).

2.4.5 Production Capacity

Installed capacity of the processing plants studied varies between 10 tons/day to 42 tons/day (Table 2.3). Presently many of the plants are not able to utilize their full installed capacity due to the deficiency of raw materials. The highest production months are August and September and the least production months are June and July. The production capacity in the preprocessing plants varies between plants (Table 2.2).

2.4.6 Effluent Treatment System

Regarding effluent treatment system, all the 20 processing plants studied are equiped with one or the other type of treatment system. 13 out of 20 processing plants have Aerobic Treatment System, five plants with Conventional Septic tank System, one with Trickling Filter System, and one with Nano Dispersion and Flocculation System (Table 2.1). However, only two out of 10 peeling shed surveyed have effluent treatment plants (Table 2.2) and one has simple aeration system.

2.4.7 Processing Technology

The processing technologies are almost similar in all the facilities visited depending up on the type of raw material. But, with respect to the market condition, the nature of processing also varies in plants. For example in some plants the fish is filleted and packed, whereas in others it is gutted or dressed and packed and in some others it is whole frozen. In the case of shrimp, IQF shrimps, de-headed, head-on, peeled deveined (PD) or peeled un-deveined (PUD), depending on the species used, whereas cephalopods, are processed as tubes and rings.

Table 2.1: Details of Seafood processing facilities selected for detailed study

Plant code	Name of the plant	Raw material	End Product *	Production capacity (ton/day)	Type of ETP *
Processing Units					
P1	Cherukattu Industries	Shrimp, Cuttlefish, Squid &Octopus (Whole)	PUD,PD,SQWC, OWC (TF)	42	CSTS
P2	Sonia Exports	Shrimp, Cuttlefish, Squid, Octopus & Fish (Whole)	PUD,PD,CFWC, SQWC,OWC,FWC (TF/PF)	16	ATS
P3	Premier Seafood Exim	Shrimp, Cuttlefish, Squid, Octopus & Fish (Whole)	PUD,PD, CFWC, SQWC,OWC (TF/PF)	20	ATS
P4	R K Exports	Shrimp, Cuttlefish, Squid, Octopus & Fish (Whole)	PUD,PD, CFWC, SQWC,OWC (TF/PF)	10	ATS
P5	Ocean Bounty Exports	Cuttlefish, Squid & Octopus	CFWC, SQWC, OWC (TF/PF)	14	ATS
P6	Moon Exports	Shrimp, Cuttlefish, Squid, Octopus & Fish (Whole/Peeled)	PUD,PD,CFWC, SQWC,OWC, FWC (TF/PF)	20	ASS
P7	SVR Exports	Shrimp, Cuttlefish, Squid, Octopus & Fish (Whole)	PUD,PD, CFWC, SQWC,OWC (TF/PF)	10	CSTS
P8	Baby Marine Exports	Shrimp	PUD & PD(IQF / BLF)	20	ASS
P9	High Seas Exim	Shrimp, Cuttlefish, Squid, Octopus & Fish (Whole)	PUD,PD,FWC,CFWC, SQWC, OWC (TF/PF)	24	ATS
P10	Kesodwala Exports	Cuttlefish, Squid, Octopus & Fish (Whole/Peeled)	CFWC,SQWC, OWC, FWC, (TF/PF)	25	ATS
P11	Lion Exports	Shrimp, Squid & Octopus	PUD,PD,SQWC, OWC (TF/PF)	10	ASS

Plant code	Name of the plant	Raw material	End Product *	Production capacity (ton/day)	Type of ETP *
P12	Abad Fisheries	Shrimp, Cuttlefish, Squid, Octopus	PUD,PD, CFWC, SQWC, OWC (TF/PF)	10	ASS
P13	Geo Aquatic	Shrimp, Cuttlefish & Fish (Whole)	PUD, PD, CFWC, FWC (TF/BLF)	13	CSTS
P14	Parayil Exports	Shrimp, Cuttlefish, Squid, Octopus & Fish (Whole)	PUD,PD, CFWC,SQWC, OWC,FWC, (TF/PF)	24	CSTS
P15	Grand Marine Exports	Cuttlefish, Squid, Octopus & Fish (Whole/Peeled)	CFWC,SQWC, OWC, FWC, (TF/PF)	24	TFS
P16	Silver Star Exports	Fish	FWC (TF)	17	CSTS
P17	Bharath Sea foods	Shrimp, Octopus & Fish	PUD,PD,OWC,FWC(PF/BLF/ IQF)	14	ATS
P18	Interseas Exports	Shrimp, Cuttlefish, Squid, Octopus & Fish (Whole)	PUD,PD, CFWC,SQWC, OWC, FWC, (TF/PF)	14	ATS
P19	Bhatson Aquatic products	Shrimp, Cuttlefish, Squid& Octopus (Whole)	PUD, PD, CFWC, SQWC, OWC (TF/PF)	31	NDFS
P20	Cochin Frozen Food Exports	Shrimp & Fish (Whole/peeled)	PUD, FWC (TF/ PF)	30	ATS

*** Types of End Products:**

BF- Blast frozen, BLF- Block Frozen, IQF- Individually Quick Frozen, PF- Plate Frozen, TF- Tunnel Frozen

CFW-Cuttlefish Whole, CFWC-Cuttlefish Whole Cleaned, FWC- Fish Whole Cleaned, GF- Gutted Fish, GO- Gutted Octopus, OWC- Octopus Whole Cleaned, PD- Peeled Deveined, PUD-Peeled Un Deveined, SQW-Squid Whole, SQWC- Squid Whole Cleaned, WS – Whole Shrimp.

***Type of Waste Water Treatment System**

NDFS-Nano Dispersion and Flocculation System; ATS- Aerobic Treatment System; CSTS - Conventional Septic tank System.

Table 2.2: Details of Peeling Sheds Surveyed

Plant Code	Name of the plant	Raw Material	End Product	Production Capacity (ton/day)	Type of ETP
PS1	T K K Marine	Shrimp (Whole)	PUD	1	Nil
PS2	Kay Kay Exports	Shrimp, Cuttlefish, Squid &Octopus (Whole)	PUD,CFWC, SQWC & OWC	13	ATS
PS3	Mangala Marine Exim India	Shrimp, Cuttlefish, Squid &Octopus (Whole)	PUD,CFWC, SQWC & OWC	12	ATS
PS4	PKD	Squid	SQWC	8	Nil
PS5	SHI Industries	Shrimp	PUD	1.5	Nil
PS6	A K Enterprises	Shrimp, Cuttlefish, Squid &Octopus (Whole)	PUD,CFWC, SQWC & OWC	13	Nil
PS7	D K Marine	Shrimp (Whole)	PUD	6	Nil
PS8	Kalapurakal	Shrimp (Whole)	PUD	1	Nil
PS9	K K H Marine	Shrimp, Cuttlefish, Squid &Octopus (Whole)	PUD,CFWC, SQWC & OWC	13	Aeration
PS10	DSS Marine	Shrimp (Whole)	PUD	1	Nil

Table 2.3: Variation in installed capacity of plants

Installed capacity (Range) (tons/day)	Number of plants
1-10	**4**
10-20	**8**
20-30	**6**
30-40	**1**
40-50	**1**

2.4.8 Quantity of raw materials handled

Quantity of raw materials processed varied from plant to plant as it mainly depends upon the procurement of raw materials (as they are seasaonal) and the market demand. Total processing capacity of all the processing plats in the study area is around 700m^3/day, but materials processed is only about 200m^3/day. Total quantity of raw materials processed in all the 20 processing units surveyed was 76304 m^3 for a year. Item wise processed, shrimp accounted for 20197m^3, cuttlefish accounted 13427m^3, squid accounted 8392m^3, octopus 6678 m^3, crab only 460 m^3 and finfish accounted 27150m^3 for a year (Table 2.4). None of the plants studied utilised its full installed capacity. Only a few of them were just up to the installed capacity. In the 10 preprocessing units surveyed total quantity of raw material peeled was 7331m^3 for a year. Item wise, shrimp accounted for 4480m^3, cuttlefish 526m^3, squid 1225m^3, octopus 1100m^3 for a year (Table 2.5). Some plants processed only shrimp and cephalopod throughout the year, (P1, P5, P11, P19), and one processsed shrimp and fish (P20). Plants which processed only one type of raw material year-round were P8 (only shrimp), P5 (only Cephalopods), P16 (only fish), and many plants processed shrimp, cephalopods and fish depending on the availability (P2, P3, P4, P6, P7, P9, P10, P13, P14, P17 and P18) and P12 processed shrimp, cephalopods and crab. P15 processed cephalopods and fish (Table 2.4)

Out of 10 pre-processing plants surveyed 5 plants peeled shrimp only (PS1, PS5, PS7, PS8 and PS10), 4 plants cleaned shrimp and cuttlefish (PS2, PS3, PS6 and PS9), and PS4 processed only cephalopod (Table 2.5).

Table 2.4: Detaills of raw materials processed by the processing facilities (July 2011- June 2012)

Plant code	Installed Capacity (ton/day)	Quantity of Raw Materials Used (Ton)							
		Shrimp	Cuttlefish	Squid	Octopus	Fish	Crab	Total/ Year	Average/ day
P1	42	3080	705	705	1266	0		5756	15.8
P2	16	810	1410	325	205	2062		4812	13.2
P3	20	1110	1590	450	330	2440		5920	16
P4	10	360	370	240	140	1710		2820	7.7
P5	14	0	1980	970	660	0		3610	10
P6	20	1135	595	540	460	1530		4260	12
P7	10	390	40	60	50	1580		2120	5.8
P8	20	665	0	0	0	0		665	1.8
P9	24	1680	1480	510	440	2020		6130	16.8
P10	25	0	670	570	250	2170		3660	10
P11	10	1510	0	780	570	0		2860	7.9
P12	10	1940	390	410	380	0	460	3580	9.8
P13	21	440	510	230	140	1880		3200	8.8
P14	24	400	720	740	360	2820		5040	13.8
P15	24	0	1420	970	650	2010		5050	13.8
P16	17	0	0	0	0	5300		5300	14.6
P17	14	975	0	0	504	257		1736	4.8
P18	14	140	544	210	57	105		1056	2.9
P19	31	4300	1003	682	216	0		6201	17
P20	30	1262	0	0	0	1266		2528	6.9
Total	396	20197	13427	8392	6678	27150	460	76304	210.58

Table 2.5: Details of raw materials handled by Peeling Sheds

Plant code	Installed capacity	Quantity of Raw material Used (Ton)						
		Shrimp	Cuttlefish	Squid	Octopus	Fish	Total/ year	Average/ day
PS1	2	640	0	0	0	0	640	1.75
PS2	13	300	120	120	120	0	660	1.8
PS3	12	360	256	560	430	0	1606	3.86
PS4	8	0	0	270	0	0	270	0.7
PS5	1.5	416	0	0	0	0	416	1.12
PS6	13	350	100	175	175	0	800	2.12
PS7	6	1550	0	0	0	0	1550	4.25
PS8	1	246	0	0	0	0	246	0.7
PS9	13	350	50	100	375	0	875	2.4
PS10	1	268	0	0	0	0	268	0.7
Total	69.5	4480	526	1225	1100	0	7331	20.1

2.4.9 Quantity of fresh water used

Major source of fresh water in the surveyed plants is bore wells and in some plants they depend up on tankers which supply fresh water. Quantity of water used in each plant varied with quantity of raw material being processed. Most seafood processors use an enormous quantity of water for cleaning plant and equipment. Therefore, water use per unit product decreases rapidly as production volume increases. Major sources of water consumption include; fish storage and transport; cleaning, freezing and thawing; preparation of brines; equipment washing; and floor cleaning. It is estimated by the Export Inspection Council (EIC) of India that at least 10 litres of water is required to process one kilogram of raw material. However the data collected revealed that most of the plants use less quantity of water, aimed for reducing the wastewater generation rate and subsequent treatment cost. Total fresh water used by the pre-processing units surveyed was 36583m^3 for a year. Average use of freshwater by each pre-processing units was 4.6 L/kg/day (Table 2.7). The amount of fresh water used in all thee 20 processing units surveyed was around 537950 m^3 for a year, and the average consumption of fresh water in each processing unit was about 7 L/kg/day (Table 2.6). Most of the processing plants have bore wells as the main source of water. Some processing plants have more than one water source. From the survey it is observed that 14 processing plants use only bore well and three use only tap water and the rest three use both bore well and tap water. Major source of water for peeling sheds are bore wells. Average use of water in the peeling sheds varying between 1000-6000 L/day. Quantity of the processed material varied between 500 – 2000 kg/day.

2.4.10 Energy consumption

Seafood processing industries consume large quantities of electrical energy. Most of the power is used for magnetic induction equipment such as electric motors (compressors for freezers, cold stores, ice making machines, water pumps, etc.) and lighting that requires magnetic ballasts, air conditioning [UNEP, 1999]. For wastewater treatment, energy is required for pumping and aerating. Energy consumption depends on various factors like age and scale of plant, the level of automation and the range of products. Electricity charge varies from ₹ 2.5 lakh to ₹ 3.8 lakh per month in the processing and exporting facilities.

2.4.11 Effluent Discharge

The details of wastewater generated by the surveyed plants are given in Table 2.6. Wastewater discharge is the most important environmental concern in the seafood industry. The whole process in the fish processing industry is water dependant and more than 90% water used became waste water**.** Fish factories in Japan have calculated water consumption to the tune of 18-60 L/kg finished products for various types of processing plants (Islam et al., 2004). Sources of effluent from seafood processing include handling and storage of raw materials prior to processing, defrosting, peeling, washing, and sorting of shrimp and cuttlefish. Gutting, scaling, portioning and filleting of fish and the washing of fish products. A study conducted by Department of Science, Technology and Environment (DOSTE), Vietnam (2003), normal cleaning of table and 4-5 m^2 of factory floor consumed about 2-3 m^3/hr of fresh water. Total effluent output from the processing units surveyed was 450057 m^3 and the average output per kilogram was 5.85 L/kg, and effluent output from 10 pre-processing units about 34635 m^3 for year, and per kilogram per day 4.36 L/kg. The volume and effluent characteristics often exhibit extreme variability. It is very difficult to estimate precisely the amount of waste discharged from each unit because of variations in daily production, water use and waste concentration values. The estimated volume of wastewater generated from all the 20 seafood processing plants is 1233 Kilolitres per Day and that of peeling shed is 94.8 Kilolitres per Day. Wastewater generation from peeling sheds is very less compared to processing plants.

Table 2.6: Quantity of fresh water used and Effluent generated in processing plants surveyed

Plant Code	Total Seafood processed (Ton)/Year	Total Fresh water used in m^3/Year	Fresh water used in L/kg/day	TotalWaste water generated (litre)/Year	Wastewater generated in L/ kg/day
P1	5756	34535	6	30504	5
P 2	4812	34050	7	27550	5.7
P3	5920	39448	6.6	35449	5.9
P4	2820	25510	9	20510	7
P5	3610	26550	7	20550	5.6
P6	4260	25559	6	20311	4.7
P7	2120	21669	9	16559	7
P8	1665	13898	8	10800	6
P9	6130	36779	5	32279	5
P10	3660	24639	6	20639	5
P11	2860	15731	5.5	12731	4.4
P12	3580	21450	5.9	17531	4.8
P13	3200	16000	5	13800	4
P14	5040	40240	7.9	35950	7
P15	5050	35249	6.9	30249	5.9
P16	5300	33899	6.3	28399	5.3
P17	1736	15416	8	13200	7.6
P18	1056	10684	10	8206	7
P19	6201	40804	6.5	32800	5
P20	2528	25840	10	22040	8.7
Total/Aver.	76304	537950	7	450057	5.8

Table 2.7: Quantity of fresh water used and Effluent generated in pre processing plants surveyed.

Plant Code	Total Seafood processed (ton)/Year	Total Fresh water used in m^3/Year	Fresh water used in L/kg/day	TotalWaste water generated (litre)/Year	Wastewater generated in L/ kg/day
PS1	640	2555	4.1	2431	3.8
PS2	660	3300	5	3036	4.6
PS3	1831	9154	5	8422	4.6
PS4	2700	1080	4	891	3.3
PS5	416	1664	4	5326	3.2
PS6	800	4000	5	3360	4.2
PS7	1550	6201	4	4185	2.70
PS8	720	2879	4	2087	2.9
PS9	875	4812	5.5	4200	4.8
PS10	268	938	3.5	697	2.60
Total / Avg	7970	36583	4.6	34635	4.35

2.4.12 Effluent Treatment Technologies

Effluent treatment technologies varied from simple screening to biological treatment methods. One or the other type of wastewater treatment and disposal mechanism is present in all the processing plant surveyed. Though all the processing plants in the study area are equipped with ETP to discharge the effluents to the adjacent water bodies as followed by the qualitative specifications of KSPCB- General Standards for Discharge of Environment Pollutants, Effluent, Gazette Notification of

MOEF May 1993, the efficiency of the system in majority of the cases is very poor because it has to handle more volume than the designed capacity; leaving no other options but to drain directly to the outlet. Even the EU approved units tend to bend the rules by under operating the installed ETP, because of the high investment costs, escalated electricity charge as well as the maintenance charge are blamed by the processors for this reason. Most widely adopted treatment system in the study area is aerobic treatment system followed by conventional septic tank system. A common effluent treatment plant is functioning in the Seafood Park, Aroor where activated sludge treatment system is followed. There is another common effluent treatment plant installed by Central Institute of Fisheries Technology (CIFT) in industrial area Aroor where Nano dispersion and flocculation system is used.

Compared to processing plants, the situation in the pre-processing units are very poor. According to MPEDA norms it is imperative for the processing facilities to have pre processing units within the facility, but most prefers to outsource it, in order to meet the labour expenses. Only few pre-processing units have any type of ETP, which is very startling. One of the reasons behind this is the number of pre-processing units varying from year to year and sometimes season to season depending on the availability of raw materials. A three tank sedimentation system is present in most of the peeling sheds. Usually wastewater is drained from these tanks to the nearest water body, and the drainage system is also very poor. The settled solid wastes at the bottom of the sedimentation tanks are cleaned periodically during night times and directly discarded to the nearby streams. Hence, the wastewater generated from the peeling shed cause greatest environmental concern.

2.5 Conclusion

Overall this study helped to understand the present scenario of the study area which is inundated by large number of preprocessing, processing and exporting and ice manufacturing industries. Processing plants used only 33% of their total available capacity, and preprocessing plants utilized only 30% of the total available capacity. August to December is the peak production time followed by January to May, June and July is the lean production time as it is the trawling ban period. Shrimps, Cephalopods (Squid, cuttlefish and Octopus) and fish are the major raw materials used. All the processing units surveyed have equipped with ETP, whereas, most of the peeling sheds lack any sort of effluent treatment system. Though these industries bring prosperity to this area by export earnings, it makes menace to this locality by obnoxious odour and alters the physical, chemical and biological characteristics of ecosystem. One of the important environmental concerns in this area from these industries is the wastewater. Unpalatable odour from the stagnated wastewater is an important issue of this area.

Groundwater of this area is greatly affected by the over exploitation of water by seafood industries as well as ice manufacturing industries. Residents of this area do not have safe and wholesome water to drink. Foul smelling and slimy water is the bane of this area. Ground water is also contaminated by leachate wastewater from seafood industries. Bore well is the primary source of fresh water in this area and it becomes highly unpalatable and contaminated by the presence of phosphates, sulphides, ammonia and salts, and pathogenic microbes.

Fig. 2.4: Iced shrimps ready for cleaning in a preprocessing unit

Fig. 2.5: Shrimp peeling

Fig. 2.6: Wastewater flowing from a peeling table

Fig. 2.7: Washing of peeled shrimps

Fig. 2.8: Wastewater flowing on the floor in a preprocessing unit

Fig. 2.9: Cephalopod processing unit

Fig. 2.10: Wastewater flowing through the drain

Fig. 2.11: A processing unit

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Chapter **3**

CHARACTERIZATION OF THE LIQUID WASTE GENERATED IN THE FISH PRE-PROCESSING AND PROCESSING CENTERS

Contents

3.1 Introduction

Seafood processing operations generate high strength wastewater, which contain organic contaminants, insoluble, colloidal and particulate form. The degree of contamination may vary according to the type of operation. A number of studies have been done on the characterization of the processed products as well as the different possible ways of utilization of fish (Gracia-Arias et al., 1994; Esp et al., 2001; Stepnowski et al., 2004) and shrimp (Jeong et al., 1991; Shahidi and Synowiecki, 1991; Benjakul and Sophanodora, 1993; Lee and Um, 1995; Chung et al., 1996; Shahidi et al., 1999; Mok and Song, 2000; Mok et al., 2000) processing by-products. Whereas, the wastewater generated from seafood processing plants, the waste load as well as the impact of the wastes in environment, have not been received enough attention since long. It is particularly important to characterize the seafood processing wastewater not only for the protection of the ecosystem but also for the sustainability of the fishery itself.

It is difficult to generalize the extent of the problem created by the wastewater as it depends on the effluent strength, wastewater discharge rate and the absorbing capacity of the receiving water body (Gonzalez, 1996). During fish evisceration and cooking, high content of COD, nutrient, oil and fats are generated in fish processing wastewater (Aguiar and Sant, 1988; Mendez et al., 1992).

Average water use during different unit operations in a shrimp canning plant indicates that peelers use as much as 58.1% of the total water consumed. Water consumption in Japanese fish factories range from 15.02 L/kg to 50.07 L/kg (1800 gal/1000 lb to 6000 gal/1000 lb) for the various types of plants. It has been reported that water use in surimi processing was 25 times the throughput. Thus, water use is 25.036 L/kg fish or 227.83 L/kg surimi (3000 gal/1000 lb fish or 27,300 gal/1000 lb surimi) (Carawan, 1991).Wastewater from fish processing and industrial fisheries is very diverse. Each plant is unique, so generalizations about water use and wastewater characteristics are difficult (Carawan, 1991). Tuna processing plants were reported to have wastewater discharge as high as 13627.4 m^3/d (3600,000 gpd). In canning of tuna, the wastewater is generated from fish thawing, washing, and eviscerating, cooling and washing of fish and cans after pre- cooking and cooking and cleaning up of washing areas (Palenzuela-Rollon, 1999). The process of cooking of squid mantle muscles generates a considerable amount of effluent can contaminate the adjacent areas if discarded into the sea (Zaidy et al., 2010). Typical effluent flow rates of the squid processing facility were estimated to be 15,000 to 20,000 gpd (gallons per day) by Park et al. (2001)

A detailed study was conducted on wastewater characterization of fish processing plant effluents under the Fraser River Estuary Management Program (FREMP, 1993). The study noticed considerable variability within and among processing plants in terms of water consumption, and effluent characteristics. The highest and lowest recorded water consumption in fish processing plants in British Columbia was 228 m^3/ tone and 2.9 m^3 /ton respectively. Contaminant concentration ranged from 128 to 2680 mg/L BOD, 316 to 3460 mg/L COD, 74 to 3640 mg/L TSS, and 0.7 to 7 mg/L ammonia. The estimated annual contaminant loadings for 1993 from all fish processing facilities to the Fraser River Estuary are 216 tonnes of biochemical oxygen demand (BOD), 380 tons of chemical oxygen demand (COD), 121 tons of total suspended solids (TSS) and 13 tonnes of ammonia. Major wastewater characteristics of concern to the seafood processing industries are pollutant parameters, sources of process waste and types of wastes. The important pollutant parameters are biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solid (TSS), fats, oils and greases (FOG) and nitrogen and phosphorus.

The most important wastewater characteristics include pH, chemical oxygen demand (COD) and biochemical oxygen demand (BOD). COD is the amount of oxygen required for oxidation of organic matter by chemical methods (Nollet, 2000). Biochemical oxygen demand is the amount of oxygen needed for oxidation of organic matter by biological action under specific standard conditions. Since, most of the seafood processing industries are located near to a water body, or the coast, they discharge their wastewaters directly onto it. When discussing the impact of this on the environment, pH and COD (or BOD) is the main factors determining the severity of the problem. High deviations of wastewater pH from neutral are capable of terminating aquatic life at the place of waste water discharge. Excessive organic matter contained in wastewater does not help aquatic life, but leads to eutrophication. In the case of food processing wastewater, COD and BOD are closely correlated and BOD levels are around 60% of those measured by COD methods (Miller et al., 2001). The COD determination is less time consuming, simpler, and more reproducible compared to that of BOD.

The main input in a processing plant are whole fresh or iced fish and shrimp, water, ice, calcium hypochlorite and other chemicals, packing materials and electricity plus liquid soap during cleaning. The output are the fresh chilled fillet exported or consumed; swim bladders, removed from fish carcasses and processed separately into valuable product; fats of red meat carcasses and fillets rejected on quality grounds are either used for human consumption or made into fish meal or silage; wastewater on varying strengths, especially from the filleting and trimming process contains fat, oil and grease (FOG) with blood, small pieces of fish and protein. The outputs of the processing industries usually contain a large bulk of waste products.
Generally, the head, shell and tail portions of shrimp are removed during processing and these account for approximately 50% of the volume of raw

materials. Increasing production of inedible parts of shrimp, such as head, shell and tail, is causing environmental problems as a result of uncontrolled dumping. The waste water from seafood processing plants contains large amounts of organic matter, small particles of flesh, breading soluble proteins, and carbohydrates. Mauldin and Szabo (1974) reported that as much as 65% of the tuna was wasted in the canning process. The average daily waste flow was over 27,000 l ton^{-1} of fish, varying from 500-1500 mg l^{-1} of BOD; 1300-3250 mg l^{-1} of COD; and 17,000 mg l^{-1} of TSS of which 40% was organic. Shells and appendages may drop off during unloading, contributing large amount of settleable solids to the waste load. Slime and body fluids and sand were also found to be part of the wastewater. Average BOD of rinse tank wastewater was 125mg l^{-1}. Scales seemed to constitute the bulk of the solids in the effluent from the wash tanks surveyed. Scott et al. (1978) and Carawan (1991) reported effluents loading at all stages of processing raw materials with a very high final bulk of wastes and the average value of waste loads.

Park et al. (2001) reported that in the commercial fish industries of Rhode Island processing of squid and several types of fin fish in fish processing facilities creates high levels of BOD in the waste water, typical BOD readings measured in the effluent of one seafood processing facility ranged 1000-5000 mg l^{-1}. Changes in processing methodologies coupled with increased landings of under-utilized species and the desire to expand production have led to dramatic increase in the volume and strength of wastewater discharged by several fish processing companies. The cleaning, separating, and packaging of under- utilized species at these companies have resulted in the generation of a complex mix of solid organic material and contaminated wastewater. For example, squid ink is released into the waste stream during processing and is known to contain high concentrations of organic matters, including highly soluble proteins, which contribute significantly to the excessive BOD loading (Shirai et al., 1997). Waldon (1991) reported that the untreated effluents from shrimp processing plants cause low dissolved oxygen (DO) concentrations in the surrounding water medium. The most important concern is that the untreated waste inputs high amounts of nutrients, such as nitrogen and phosphorous, which contribute to the eutrophication of receiving water in the surrounding area. Seafood-processing wastewater characteristics that raise concern include pollutant parameters, source of process waste, and types of wastes. In general, seafood-processing wastewater can be characterized by its physicochemical parameters, organics, nitrogen, and phosphorus contents and other dissolved heavy metals present in trace quantities. Effluents from fish and crustacean processing plants are generally characterized by high concentrations of nutrients; high levels of nitrogen content found as ammonia (NH_3-N; 29 to 35 $mg.L^{-1}$), high total suspended solids (0.26 to 125,000 $mg.L^{-1}$), increased biochemical oxygen demand (10 to 110,000 $mg.L^{-1}$) and chemical oxygen demand (496 to 140,000 $mg.L^{-1}$) and by the presence of sanitizers. (AMEC Earth and Environmental Limited 2003).

3.2 By-product recovery from seafood processing effluent

Though some authors have observed that reuse and recycling of waste materials are difficult to apply in food processing industries (Mc Donald et al., 1999 and Asbjorn, 2004) suggested that better returns can be obtained by utilizing seafood effluent by-products for industrial use and human consumption such as biopolymers; enzymes and bioactive peptides. The following table (Table 3.1) displays seafood processing effluent utilization options proposed by different researchers.

Table 3.1: Seafood waste utilization options

By product	References
Protein recovery	Perez-Galvez et al., 2011; Afonso and Borquez, 2002
Enzymes and pigments	Stepnowski et al., 2004[a&b], Dewitt and Morrissey et al., 2002
Aroma compounds	Walhaa et al., 2011; Cros et al., 2004
Oil recovery	Sala, 2012; Hsieh et al., 2005

3.3 Waste Water Characteristics

3.3.1 Physicochemical characters

Although the volume and characteristics of shrimp and fish processing effluents often exhibit extreme variability, waste production in seafood processing industries is usually high in volume. The BOD may be as low as 100 mg l^{-1} to as high as 200,000 mg l^{-1}. Suspended solids may be found in concentration as high as 120,000 mg l^{-1}. The waste may be alkaline (pH 7.8) or acidic (pH 6.4). Nutrients such as nitrogen and phosphorous may be absent or they may be present in quantities in excess of those necessary to promote suitable environmental conditions for biological treatment. Carawan et al. (1986) reported a BOD of 200-1000 mg l^{-1}, COD of 400-2000 mg l^{-1}, TSS of 100-800 mg l^{-1}, and FOG of 40-300 mg l^{-1} from seafood processing plant wastes.

Seafood processing wastewater was noted to sometimes contain high concentrations chlorides from processing water and organic nitrogen (0-300 mg l^{-1}) from processing water and brine solutions (Flick and Martin, 2012).

3.3.1.1 pH

pH is an important parameter that determines the contamination of wastewater and the need for pH adjustment for biological treatment of the wastewater. Usually it is close to neutral in seafood processing effluents. For example, a study found that the average pH of effluents from blue crab processing industries was 7.63, with a standard deviation of 0.54; for non-Alaska bottom fish, it was about 6.89 with a standard deviation of 0.69 (Carawan et al., 1979). The results obtained from four different fisheries from British Columbia showed pH in the range of 5.7- 7.4 with an average pH of 6.48 (Technical Report Series FREMP, 1993) The pH levels generally reflect the decomposition of proteinaceous matter and emission of ammonia compounds (Gonzalez, 1996).

3.3.1.2 Solid Contents

Solid contents in the seafood effluents can be divided into dissolved solids and suspended solids. Suspended solids create more problem as they affect the aquatic life by interrupting the sun light to reach the bottom dwelling flora and the food chain. The major types of solid wastes which contribute significantly to the suspended solid concentration in seafood processing effluents are blood, offal products, entrails, fins, fish heads, shells, skins and fine flesh.

In general, fish processing wastewater contain high levels of suspended solids which are mainly proteins and lipids (Palenzuela –Rollon et al., 2002). Carawan et al. (1979) observed that in tuna processing the average value of total solids was 17,900 mg/L of which 40% was organic. The fish condensate has high volatile solids (VS) consisting of trimethyl amine (TMA) and volatile fatty acids (VFA). The wastewater characteristics from fish processing units depend on the composition of raw fish, the unit processes, source of processing water and additives used such as brine, oil for the canning process (Palenzuela –Rollon., 1999)

3.3.1.3 Odor

Odor is an important issue in relation to public perception and acceptance. Decomposition of organic matter produce odor in seafood processing industries, which emits volatile amines and ammonia. Even though it is harmless, it may affect the public life by inducing stress and sickness.

3.3.1.4 Organic Content

Seafood processing operations generates a high strength wastewater and the degree of contamination varied according to the type of operation. Stone et al. (1981) reported that the amount of water used for each unit production decreases as daily production increases. The same was observed and reported by Nova Tec Consultants Inc. and EVS Environment Consultants (1994).

In one study, the fluming flow was estimated to be 834 L/tone of fish with a suspended solids loading of 5000 mg/L. The solids consisted of blood, flesh, oil, and fat (Carawan et al., 1979). The above figures vary widely. Other estimates listed herring pump water flows of 16 L/sec with total solids concentration of 30,000 mg/L and oil concentration of 4000 mg/L. The boat's bilge water was estimated to be 1669 L/ton of fish with a suspended solids level of 10,000 mg/L (Carawan et al., 1979). Stickwater comprises the strongest wastewater flows. The average BOD_5 value for stick water has been listed as ranging from 56,000 to 112,000 mg/L, with average solids concentrations, mainly proteinaceous, ranging up to 6%. The fish-processing industry has found the recovery of fish soluble from stick water to be at least marginally profitable. In most instances, stick water is now evaporated to produce condensed fish soluble.

The characteristics of wastewater are found to be greatly affected by the raw materials used in the processing plants and the quality of the raw materials to be processed has also been found to vary as a function of time (Omil et al., 1995). The high strength wastewaters such as the one generated during the fish meal production are often known to be diluted with cooling waters from the overall process, prior to disposal (Alfonso and Borquez, 2002).

The degree of pollution due to wastewater depends on several parameters. The most important factors are the types of operation being carried out and the type of seafood being processed. (Carawan et al., 1979) reported on an EPA survey with BOD_5, COD, TSS, and fat, oil and grease (FOG) parameters. Bottom fish was found to have a BOD_5 of 200–1000 mg/L, COD of 400–2000 mg/L, TSS of 100–800 mg/L, and FOG of 40–300 mg/L. Fish meal plants were reported to have a BOD_5 of 100–24,000 mg/L, COD of 150–42,000 mg/L, TSS of 70–20,000 mg/L, and FOG of 20–5000 mg/L. The highest numbers were representative of bail water only. Tuna plants were reported to have a BOD_5 of 700 mg/L, COD of 1600 mg/L, TSS of 500 mg/L, and FOG of 250 mg/L. Several methods are used to estimate the organic content of the wastewater. The two most common methods are biochemical oxygen demand (BOD) and chemical oxygen demand (COD).

3.3.1.5 Biochemical Oxygen Demand

Biochemical Oxygen Demand is the amount of oxygen required for the oxidation of organic matter by aerobic microbes. It estimates the degree of contamination in a water body. In seafood-processing effluent, BOD primarily originates from carbonaceous compounds which are used as substrate by the aerobic microorganisms; and secondarily from nitrogen containing compounds that are normally present in seafood-processing wastewaters, such as proteins, peptides, and volatile amines. Wastewaters from seafood-processing operations can be very high in BOD_5. A literature in seafood processing operations shows BOD_5 production of one to 72.5 kg of BOD_5 per ton of product. Most of the BOD usually comes from hold water and from the butchering process (Technical Report Series FREMP, 1993). Fish canning industries have a high concentration of organic pollutants in the range 10,000-50,000 mg/L (Mendez et al., 1992). White fish filleting processes typically produce 12.5–37.5 kg BOD_5 for every ton of product. The BOD is generated primarily from the butchering process and from general cleaning, while nitrogen originates predominantly from blood in the wastewater stream (Environmental Canada, 1994).

3.3.1.6 Chemical Oxygen Demand

Chemical Oxygen Demand is an alternative method for measuring the organic content in a wastewater. It is an important pollutant parameter for the seafood industry. The COD of an effluent is usually higher than the BOD_5, as the number of compounds that can be chemically oxidized is greater than those that can be degraded biologically, Hence, it is common practice to correlate BOD_5 COD and then use the analysis of COD as a rapid means of estimating the BOD_5 of wastewater. Depending on the types of seafood processing, the COD of wastewater can range from 150 to about 42,000 mg/L. Effluent BOD- COD ratios varied widely within and among processing plants ranging from 1.1:1 to 3:1 (Technical Report Series FREMP, 1993). Carawan et al. (1979) observed that the BOD (500-1500 mg/L) of tuna waste was only 40 % of the COD (1300-3250 mg/L) value. As reported by Del Valle and Aguileria (1990) fish meal blood water contributed the highest COD value (93,000 mg/L) among all the processes.

3.3.1.7 Fats, Oil, and Grease

Fats, oil, and grease (FOG) is an important parameter in the seafood-processing effluent. The FOG should be removed from wastewater because it usually floats on the water's surface and affects the oxygen transfer to the water; it is also objectionable from an aesthetic point of view. The FOG may also cling to wastewater ducts and reduce their capacity in the long term. Around 60% of the oil and grease originates from the butchering process (Nova Tec, 1994). Rest of the oil and grease is generated during fish canning and fish processing operations (Gonzalez, 1996). Carawan et al. (1979) reported the FOG values for herring, tuna, salmon and catfish processing were 60-800 mg/L, 250 mg/L, 20-550 mg/L and 200 mg/L, respectively.

3.3.1.8 Total ammonia nitrogen (TAN)

TAN is composed of NH_3-N (unionized ammonia) and $NH4^+$ (ionized ammonia) (Losordo et al., 1992; Masser et al., 1992) it is the unionized form that is most toxic to aquatic organisms as it can readily diffuse through cell membranes and is highly soluble in lipids (Chin and Chen, 1987). Ammonia, formed only at high pH values (pH>8.5) is extremely toxic to fish and other aquatic life at high concentration (> 2.0 mg/l N) (DFIED, 1999).

3.3.1.9 Nitrogen and Phosphorus

The breakdown of organic contaminants in the effluent resulted to release nitrogen and phosphorus. If they are in excess they may cause proliferation of algae and affect the aquatic life in a water body. High nitrate levels in waste effluents could also contribute to the nutrient load of the receiving waters and so contribute to eutrophication effects, particularly in fresh water (Fried, 1991; OECD, 1982; WRC, 2000). However, their concentration in the seafood-processing wastewater is minimal in most cases (Gonzalez, 1996). It is recommended that a ratio of N to P of 5: 1 is recommended for proper growth of the biomass (Eckenfelder, 1980; Metcalf and Eddy Inc., 1979). The high nitrogen levels are likely due to the high protein content (15-20% of wet weight) of fish and marine invertebrate (Sikorski, 1990). Phosphates are undesirable anions in receiving waters and act as the most important growth limiting factor in eutrophication and result in a variety of adverse ecological effects (OECD, 1982, WRC 2000). Phosphorus also partly originates from the fish, but can also be introduced with processing and cleaning agents (Intrasungkha et al., 1999). Sometimes high ammonia concentration is observed due to high blood and slime content in wastewater streams. The overall ammonia concentration ranged from 0.7mg/L to 69.7mg/L (Technical Report Series FREMP, 1993). The degree of ammonia toxicity depends primarily on the total ammonia concentration and pH.

3.4 Heavy metals

Metals having high density and toxic even at low quantity are known as heavy metals. Eg. Arsenic (As), Lead (Pb), Mercury (Hg), Cadmium (Cd), Chromium (Cr), Thallium (Tl) etc. By the formation of toxic soluble compounds certain heavy metals become toxic, whereas some metals without any biological role become poisonous in specific forms. However, any amount of Pb can result in deleterious effect. It enters the body through respiration, ingestion and skin.

All the samples were tested for the presence of heavy metals such as Cadmium, Lead and Mercury, which are highly toxic and dangerous to living organisms. These heavy metals have greater affinity for sulphur and disarray enzyme function by forming bonds with sulphur groups in enzymes. These heavy metals can bind to cell membrane and impede transport process through the cell wall.

Lead (Pb)

Lead is a cumulative poison which comes from leaded gasoline, used to be a major source of atmospheric and terrestrial lead, ultimately end up in the aquatic environment. It enters the fish body from its polluted habitat.

Mercury (Hg)

Mercury is pervasive in the environment from natural resources can enter the aquatic environment via dissolution and biological process. Mercury in the form of Methyl Mercury is highly toxic to organisms, because it cannot be excreted and acts as a cumulative poison. Because of human health hazards of consuming mercury-contaminated seafood, several nations are forced to make regulations and guidelines for allowable seafood mercury levels. Drinking water standard of mercury is 1µg/L.

3.5 Microbiological Characters

Health risk associated with organic wastes is the presence of high concentrations of pathogenic organisms and their potential to spread diseases (WHO, 2010). Bagge-Raven et al. (2003) demonstrated that the processing equipment of food industries harbors a microbial ecosystem both during production and after cleaning and disinfection. The micro flora is partly a reflection of raw material used (fish and shrimp) and partly a reflection of preservation parameters used in the products (e.g., NaCl and acid). Karunasagar and Karunasagar (2000) reported the occurrence of food borne human pathogens such as *Listeria* spp. in fresh fish as well as processed fishery products.

3.6 Materials and Methods

This study envisages a preliminary characterization of seafood processing plant effluents through organic and inorganic analysis. Because of basic similarities among the processing plants, it was decided to select four typical plants at random and the samples were designated as shrimp pre-processing (Sample A), Cephalopod (squid, cuttlefish and octopus) processing (Sample B), fish filleting (Sample C) and shrimp processing (Sample D). Samples were collected in plastic containers cleaned with detergent rinsed with tap water and finally washed with deionised water before usage and carefully collected to avoid any contamination by foreign materials and brought to laboratory and stored at $4^{o}C$ in a refrigerator. Samples for microbiological analysis were collected in autoclaved sterilized bottles to avoid contamination. Samples collected on a monthly basis for six months and analyzed physicochemical as well as microbiological characters. The samples collected were analyzed for pH, BOD, COD, Nitrate and Nitrite, Ammonia, Total Solids and Total Suspended Solids, heavy metals like Hg, Cd and Pb, total coliform and fecal coliform. All tests were performed in accordance with procedures developed by American Public Health Association, (APHA) 1995.

3.6.1 pH

pH was measured by using digital pH meter

3.5.2 Estimation of alkalinity

Alkalinity of the effluent samples were measured by titrating with concentrated hydrochloric acid (APHA, 1995). Titration up to pH 8.3 or decolorisation of phenolphthalein indicator indicate complete neutralization of OH and half of the CO_3, the value is called phenolphthalein alkalinity, whereas at pH 4.5 or sharp change from yellow to pink with methyl orange indicator, indicate total alkalinity (complete neutralization of OH, CO_3 and HCO_3). Alkalinity is commonly expressed in mg $CaCO_3$/L.

An aliquot of 100 mL sample was taken in a conical flask and added 3 drops of phenolphthalein indicator. If there has been no colour produced, the phenolphthalein alkalinity turns out to be zero. If pink color gets developed it is titrated against 0.1 N hydrochloric acid (8.3 mL of concentrated hydrochloric acid in 1L distilled water) till the colour disappears or pH 8.3 is attained. Subsequently, an aliquot of 3 drops of methyl orange was added to the same flask, and continued titration till pH dropped to 4.5 or the yellow colouration changed to orange. The volume of hydrochloric acid used was noted. In case the pink coloration did not appear after addition of phenolphthalein, the estimation of methyl orange alkalinity was continued.

Calculation

Phenolphthalein alkalinity (mg/L $CaCO_3$) = (A× 1000)/ mL sample

A = Pink to colorless end point

Total alkalinity of sample in mg $CaCO_3$/L

= (Normality of HCL × volume of HCL consumed /volume of sample taken) ×50 × 1000

Phenolphthalein and total alkalinities are determined. Three types of alkalinity can be calculated from Table 3.2.

Table 3.2: Relationship between hydroxide (OH^-), carbonate (CO_3^-) and bicarbonate (HCO_3 -) alkalinities

Result of titration	OH alkalinity	CO_3 alkalinity	HCO_3 alkalinity
P = 0	0	0	T
P < ½ T	0	2P	T – 2P
P = ½ T	0	2P	0
P > ½ T	2P – T	2 (T – P)	0
P = T	T	0	0

P = Phenolphthalein alkalinity, T = Total alkalinity

3.6.3 Estimation of dissolved oxygen (Iodometric method) (APHA, 1995)

Dissolved oxygen present in the sample rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide to hydroxide of higher valance state which gets precipitated as brown hydrated oxidizes after the addition of sodium hydroxide and potassium iodide. Upon acidification, manganese reverts to divalent state and liberates iodine from potassium iodide equivalent to dissolved oxygen content in the sample. The liberated iodine is titrated against sodium thiosulphate using starch as an indicator. If no oxygen is present, a pure white precipitate of Mn (OH) forms when manganous sulphate and alkali reagents are added to the sample. If oxygen is present the divalent Mn (II) is oxidized to Mn (IV) and precipitate as brown hydrated oxide.

Effluent samples were collected in BOD bottles taking care to avoid air bubbles getting trapped. An aliquot of 1 mL Winkler A (480 gm manganous sulphate dissolve din 1 L distilled water) followed by Winkler B (500g sodium hydroxide and 10g sodium azide dissolved in 1L distilled water) were added immediately after collection and placed stopper carefully excluding air bubbles, and mixed the solution by inverting the bottles repeatedly. The stopper of the bottle was carefully removed and added 1mL concentrated sulphuric acid and mixed by gentle inversion until the precipitate was completely dissolved. An aliquot of 50mL of the preparation was titrated against 0.025N sodium thiosulphate (6.205 g dissolved in 500 mL distilled water and added 0.4g solid sodium hydroxide), using starch as the indicator until the blue color turned to colorless.

Calculatioin

Oxygen content of the sample (mg/L)

$$= (8 \times \text{volume of titrant} \times \text{normality of titrant} \times 1000)/\text{volume of sample}.$$

3.6.4 Estimation of Ammonia (Solorzano, 1969)

Ammonia reacts in moderately alkaline solution with hypochlorite to monochloramine which, in the presence of phenol, catalytic amounts of nitroprusside ions and excess hypochlorite, gives indophenols blue. The formation of monochloramine requires a pH between 8 and 11.5. at a pH higher than 9.6, precipitation of Mg and Ca ions as hydroxides and carbonates occurs in seawater. However, these ions can be held in solution by complexing them with citrate.

An aliquot of 10 mL sample was taken in a test tube, added 0.4 mL phenol solution (20 g of crystalline phenol dissolved in 95%V/V ethyl alcohol), 0.4 mL sodium nitropruside (1g dissolved in 200 mL distilled water), and 1.0 mL oxidizing solution [alkaline reagent (100 g sodium citrate and 5 g sodium hydroxide dissolved in 500 mL distilled water) and sodium hypochlorite 4:1 ratio]. Absorbance was taken at 640 nm after 1hr incubation at room temperature. A series of standards (4.714 mg ammonium chloride dissolved in 100 mL double distilled water gave 10 micro gram /mL ammonia – nitrogen) were prepared and the factor value was calculated.

$$\text{Ammonia nitrogen in mg/L} = \text{Factor Value} \times \text{Absorbance of the sample}$$

$$\text{Factor Value} = \text{Concentration of standards/ absorbance.}$$

3.6.5 Estimation of Nitrite and Nitrate (Bendschneider and Robinson, 1952)

Under acidic condition (pH 2.0 to 2.5) nitrite ion (NO_2-) as nitrous acid (HNO_3) react with sulphanilamide to form diazonium salt, which combine with N-(1-napthyl)-ethylene diamine dihydrochloride (NED dihydrochloride) to form a bright coloured pinkish red azo dye. The colour produces is directly proportional to the amount of nitrite present in the sample.

Nitrate is determined by converting nitrate to nitrite using a mixture of phenol- sodium hydroxide and copper sulphate – hydrazine sulphate. Reagents are added and incubated at dark for 18 hours, added acetone and complexed with sulphanilamide and NED.

3.6.5.1 Nitrite analysis

An aliquot of 10 mL sample was taken in a test tube, added 0.2mL sulphanilamide (5 g dissolved in a mixture of 50 mL concentrated hydrochloric acid and 450 mL distilled water) and 0.2mL of NED (0.5g dissolved in 500 mL distilled water). The absorbance was taken after 8 minutes at 543 nm. Series of standards (4.925 mg sodium nitrite dissolved in 100 mL gave 10 microgram/mL) were prepared and calculated the factor value.

3.6.5.2 Nitrate analysis

To the 10 mL sample added 0.4 mL phenol-sodium hydroxide solution [This solution was prepared by mixing phenol solution (46 g dissolved in 1L distilled water) and sodium hydroxide (30 g dissolved in 2 L distilled water) at 1:1 ratio] and 0.2 mL hydrazine sulphate – copper sulphate solution [this solution prepared by mixing of hydrazine sulphate (14.5 g hydrazine sulphate dissolved in 1L distilled water) and copper sulphate (0.1g copper sulphate dissolved in 1L distilled water) at 1:1 ratio], incubated in dark for 18- 24 hours. After incubation 0.4 mL acetone, 0.2 mL sulphanilamide and 0.2 mL NED were added. Absorbance was measured after 8 minutes at 543 nm. A series of standards (6.0707 mg sodium nitrate dissolved in 100 mL gave 10 µg/mL Nitrate-nitrtogen) was prepared and calculated the factor value.

Calculation

Concentration of nitrate in sample in mg/L =[(x-y) × 100/efficiency

Where x = Absorbance of nitrate × Factor value of nitrate

Y = corresponding concentration of nitrite

Efficiency = (A/B) × 100

Where A = Observed concentration of standard (absorbance × factor value of nitrite)

B = Original concentration of standard prepared.

Efficiency measures the percentage of nitrate converted into nitrite.

3.5.6 Estimation of Biochemical Oxygen Demand (BOD_5) (APHA, 1995)

Biochemical oxygen demand is defined as the amount of oxygen required by bacteria in decomposing organic material in a sample under aerobic conditions at 20^{o} C over a period of 5 days.

Dilution method

1. Preparation of dilution water

   a) Aeration – Aerated 1 L distilled water by bubbling compressed air for 1 day to attain dissolved oxygen saturation. Tried to maintain temperature near 20^{o} C

b) pH - Neutral pH (7.2).

c) Addition of nutrients – 1mL each of phosphate buffer (8.5 g potassium dihydrogen phosphate, 33.4 g disodium hydrogen phosphate and 1.7 g ammonium chloride dissolved in 1L distilled water), magnesium sulphate (22.5 g dissolved in 1L distilled water) and ferric chloride (0.25 g dissolved in 1L distilled water) solution were added to 1 litre of dilution water and mixed well.

Dilution method (without seeding)

Samples were diluted in standard dilution water in a graduated cylinder of 1000 mL capacity and thoroughly mixed together. Transferred the diluted sample into 4 labeled BOD bottles and closed immediately. One bottle was used for determination of the initial dissolved oxygen and other 3 bottles were incubated at 20^o C for 5 days. After incubation 1mL Wrinkler A and 1 mL Wrinkler B reagents were added into the bottle by inserting the calibrated pipette just below the surface of the liquid, brownish-orange cloud of precipitate or floc appear. When this floc settled to the bottom 2 ml conc. H_2SO_4 was added, stopped and inverted several times to dissolve the floc. Titrate 50 ml sample with sodium thiosulphate to a pale straw colour, and then 2 ml. of starch solution was added so a blue colour was formed. Titration was continued until the sample turned clear.

Initial and final dissolved oxygen contents of the samples were determined.

Calculation

BOD mg/L = D1-D2/P

Where

D1	= DO of the sample bottle on 0 day
D2	= DO of the sample after 5 days
P	= decimal fraction of the dilution water used
P	= volume of wastewater/ volume of wastewater + dilution water

3.6.7 Estimation of Total solids

Total Solids (TS) is the measure of all kinds of solids i.e. suspended, dissolved and volatile solids. Total solids can be determined as the residue left after evaporation at 103 to 105 ^oC of the unfiltered sample.

A measured volume of unfiltered, well mixed sample was poured in a preheated and weighed evaporating dish (dried at 103 to 105^oC for 1 hour), kept in the hot air oven at 103^oC to 105^oC for 2 hours, cooled in the desiccator and the final weight was taken.

Calculation

Total solids mg/L = Weight of final (Wf) – Weight of initial (Wi) × 1000/volume of sample, mL

3.6.8 Estimation of Total Suspended Solids (TSS)

It is a major parameter used to evaluate the strength of the effluent and to determine the efficiency of wastewater treatment unit.

Wetted the filter paper by double distilled water, stirred the sample and pipetted a measured volume of sample onto the filter, washed with

3- successive 10 mL volume of distilled water and continued suction for about 3 minutes. Removed the filter paper and dried for 1 hour at 103 to $105^{o}C$. Cooled in a desiccator and weighed.

Calculation

Total suspended solids mg/L = (Wf – Wi) × 1000/ volume of sample

3.6.9 Estimation of Chemical Oxygen Demand (COD) (Open Reflux Method) (APHA 1995)

Chemical oxygen demand is defined as the amount of a specific oxidant that reacts with the sample under controlled conditions, the quantity of oxygen consumed is expressed in terms of oxygen equivalence.

Open reflux method

Most type of organic matter are oxidized by a boiling mixture of chromic acid and sulphuric acid. A sample is refluxed in strongly acidic solution with a known excess of potassium dichromate. After digestion, the remaining unreduced potassium dichromate is titrated with FAS to determine the amount of potassium dichromate consumed and the oxidizable matter is calculated in terms of oxygen equivalent.

Pipetted out 50mL of the blended sample into a 500mL refluxing flask, added 1g $HgSO_4$, several glass beads and very slowly added 5mL of H2SO$_4$ with mixing to dissolve $HgSO_4$, cooled while mixing to avoid possible loss of volatile materials. Added 25mL of 0.04167M $K_2Cr_2O_7$ and mixed well. Attached the flask to the condenser and cooling water turned on, added remaining 70 mL H_2SO_4 through the open end of the flask, swirled and mixed well and refluxed for 2 hours. Cooled to room temperature and titrated excess of potassium dichromate with FAS using ferroin indicator. The color changed from blue green to reddish brown that persisted for 1 minute.

Calculation

COD as mg O_2/L = (A-B) × M × 8000/mL of sample

Where

A = mL of FAS used for blank

B = mL of FAS used for sample

M = Molarity of FAS

8000 = milli equivalent weight of Oxygen × 1000 mL/L

3.6.10 Estimation of Chloride

The chloride is titrated against $AgNO_3$ using $KCrO_4$ as indicator. The end point is reddish brown color due to formation of $AgCrO_4$.

Directly titrated the samples in the pH range 7-10, 50 mL of the sample is titrated against 0.0141 N. $AgNO_3$, using $KCrO_4$ as indicator. Colour changed from yellow to reddish brown.

Calculation

MgCl/L = V × N × 35.45 × 1000/ sample volume

Where

V = volume of titrant

N = normality of $AgNO_3$

3.7 Heavy Metal Analysis

All the samples were analyzed with ICP-AES system

3.8 Microbiological Analysis (APHA, 1995)

The examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly functioning sewage treatment systems (Palanisami, et al., 2005). In order to determine whether water has been contaminated by fecal material, a series of tests are used to demonstrate the presence or absence of coliforms. The coliform group is comprised of Gram-negative, nonspore-forming and aerobic to facultatively anaerobic rods, which ferment lactose to acid and gas. The only true fecal coliform is *E. coli,* which is found only in fecal material from warm-blooded animals. *E. coli* is a normal inhabitant of the intestinal tract and is not normally found in fresh water. This pathogen can transmit through water, food and contact with animals or persons. Therefore, if it is detected in water, it can be assumed that there has been fecal contamination in the water.

The Most Probable Number (MPN) technique was used for enumeration of Coliforms, fecal Coliforms and fecal streptococci for all samples according to standard methods (APHA, 1995). MPN is a procedure to estimate the population density of viable microorganisms in a test sample. It is based upon the application of the theory of probability to the numbers of observed positive growth responses to a standard dilution series of sample inoculums placed into a set number of culture media tubes.

3.8.1 MPN Procedure

Total coliforms can be detected and enumerated in the multiple-tube technique. In the multiple-tube method, a series of tubes containing a suitable selective broth culture medium (lactose-containing broth, such as MacConkey broth) is inoculated with test portions of a water sample. After a specified incubation time at a given temperature, each tube showing gas formation is regarded as "presumptive positive" since the gas indicates the possible presence of coliforms. However, gas may also be produced by other organisms, and so a subsequent confirmatory test is essential. The two tests are known respectively as the presumptive test and the confirmatory test. For the confirmatory test, a more selective culture medium (brilliant green bile broth) is inoculated with material taken from the positive tubes. After an appropriate incubation time, the tubes are examined for gas formation as before. The most probable number (MPN) of bacteria present can then be estimated from the number of tubes inoculated and the number of positive tubes obtained in the confirmatory test. Using specially devised statistical tables. This technique is known as the MPN method

3.8.2 Presumptive Test

The first step of the MPN procedure for fecal coliform testing is called the presumptive test. In this test, samples or serial sample dilutions are inoculated into a series of fermentation tubes. The fermentation tubes are then incubated at 35 +/-0.5°C. The tubes are observed at the end of 24 and 48 hours for gas production. Any tube showing gas production during this test indicates the possible presence of coliform group bacteria and is recorded as a positive presumptive tube. All positive presumptive tubes are transferred to EC broth fermentation tubes to confirm the presence of fecal coliform bacteria.

1) Labeled three single-strength lactose broth tubes "0.I," labeled another three tubes "I," and labeled the three double strength broth tubes "10."
2) Inoculated each 0.1 tube with 0.1ml of water sample.
3) Inoculated each 1 tube with 1.0 ml of water sample.
4) Inoculated each 10 tube with 10 ml of the water sample.
5) Incubated the tubes for 24 to 48 hours at 35°C.

3.8.3 Fecal Coliform Confirming Test

In the confirming test procedure for fecal coliform bacteria, the positive presumptive cultures are transferred to EC broth, which is specific for fecal coliform bacteria. Any presumptive tube transfer which shows gas production after 24 (+/-2) hours incubation at 44.5°C (+/-0.2°C) confirmed the presence of fecal coliform bacteria in that tube and was recorded as positive confirmed tube.

1) Paired each positive presumptive fermentation tube with a fermentation tube containing EC broth. Marked each EC tube to match its paired presumptive tube.
2) Using a sterile transferred loop, transfer a portion of the liquid from each presumptive tube to its paired EC broth fermentation tube.

3) Discarded the positive presumptive tubes after transferring using appropriate safety precautions.

4) Placed all of the inoculated EC broth tubes in a water bath incubator maintained at 44.5° +/-0.2°C.

5) Incubated the EC broth tubes for 24 (+/-2) hours.

6) Removed the tubes from the water bath, shaken gently and inspected for gas production.

7) Recorded all fermentation tubes showing gas production as positive on the test data sheet.

8) Calculated the test results and recorded as Most Probable Number (MPN)/100 mL.

9) Discarded the fermentation tube contents using appropriate safety precautions.

a) Complete Test

Inoculum from each positive confirmatory tube were streaked on Mac Conkey agar plate and incubated at 44^{o}C for 24-48 hours. Observed dark pink colored bacterial colonies.

b) E-coli identification test

Took a loopful of bacterial colony from the Mac Conkey agar plate and evenly spread it on a slide and stained with crystal violet, fixed with iodine, added 2-3 drops of acetone, added saffronine after 45 seconds. Observed under microscope, pink coloured rod shaped *E.coli* could seen.

3.8.4 Calculation of Most Probable Number (MPN)/100 mL

To increase the statistical accuracy of this type of test, more than one broth tube can be inoculated from each dilution. Standard MPN procedures use a minimum of 3 dilutions and 3, 5 or 10 tubes per dilution. The statistical variability of bacterial distribution is better estimated by using as many tubes as possible or practical. After incubation, the pattern of positive and negative tubes is noted, and a standardized MPN Table is consulted to determine the most probable number of organisms (causing the positive results) per unit volume of the original sample. The calculation of the MPN test results requires the selection of a valid series of 3 consecutive dilutions. The number of positive tubes in each of the three selected dilution inoculations is used to determine the MPN/100 mL. In selecting the dilutions to be used in the calculation, each dilution is expressed as a ratio of positive tubes per tubes inoculated in the dilution. There are several rules to follow in determining the most valid series of dilutions. Select the highest dilution showing all positive results (no lower dilution showing less than all positive) and the next two higher dilutions.

After selecting the valid series, the MPN/100 mL is determined by matching the selected series with the same series on the MPN reference chart. If the selected series does not match the sample dilution series at the top of the MPN reference chart, the results must be calculated using the following formula:

MPN/100 mL = MPN from chart x (mL sample for first column of chart/mL sample in first dilution of the selected series)

3.9 Results and Discussion

The data collected for the study of physicochemical parameters were compiled and analyzed statistically using Student's 't' test for comparison of means of two samples and Two Factor ANOVA technique for comparison of the effect of various parameters under study between centers and between months.

Table 3.3: Effect of various parameters under study between samples and between months.

	Source of variation	Ss	Df	Ms	F	p-value
pH	Total	3.9854	23			
	Month	0.4103	5	0.0821	0.828	$p>0.05$
	Processing centers	2.0881	3	0.6960	7.024	$P<0.001$*
	Error	1.4870	15	0.0991		
TSS	Total	1748818.95	23			
	Month	335380.65	5	67076.1305	2.289	$p>0.05$
	Processing centers	973779.91	3	324659.97	11.082	$P<0.001$*
	Error	439458.39	15	29297.23		
TS	Total	33890721.92	23			
	Month	13875350.38	5	2775070.08	3.865	$P<0.05$*
	Processing centers	9245720.22	3	3081906.74	4.292	$P<0.01$*
	Error	10769651.32	15	717976.76		
NH_3	Total	8285.8847	23			
	Month	1375.2532	5	275.0506	6.013	$P<0.001$*
	Processing centers	224.4735	3	74.8245	1.636	$p>0.05$
	Error	686.1580	15	45.7439		

	Source of variation	Ss	Df	Ms	F	p-value
NO_2	Total	48.9384	23			
	Month	2.7386	5	0.5477	0.476	P>0.05
	Processing centers	28.9275	3	9.6425	8.374	P<0.001*
	Error	17.2723	15	1.1515		
NO_3	Total	0.1363	23			
	Month	0.0274	5	0.0055	0.870	p>0.05
	Processing centers	0.0145	3	0.0048	0.769	P>0.05
	Error	0.0944	15	0.0063		
O &G	Total	164.0994	23			
	Month	33.2278	5	6.6456	1.312	p>0.05
	Processing centers	54.8997	3	9.1500	1.807	p>0.05
	Error	75.9719	15	5.0648		
BOD	Total	13460537.63	23			
	Month	4076618.38	5	815323.68	4.468	P<0.01*
	Processing centers	6646644.74	3	2215548.26	12.141	P<0.001*
	Error	2737274.46	15	182484.96		
COD	Total	15998749.96	23			
	Month	5317898.21	5	1063579.64	5.307	P<0.001*
	Processing centers	7674561.46	3	2558187.15	12.764	P<0.001*
	Error	3006289.29	15	200419.29		
Cl	Total	346737.83	23			
	Month	117177.33	5	23435.47	1.700	P>0.05
	Processing centers	22718.17	3	7572.72	0.549	P>0.05
	Error	206842.33	15	13789.49		
Alkalinity	Total	199974.5	23			
	Month	51605.5	5	10321.10	1.862	P>0.05
	Processing centers	65223.5	3	21741.17	3.922	P<0.01*
	Error	83145.5	15	5543.03		

3.9.1 pH

pH serve as one of the important parameters because it may reveal contamination of a wastewater body or indicate the need for pH adjustment for biological treatment of the wastewater. Average pH was 7.5 in both samples C and D, 7.4 in sample B and 6.8 in sample A. (Fig.3.1). Variation in pH values of effluent can affect the rate of biological reactions and survival of various microorganisms. Generally, pH of seafood effluents is close to neutral. For example, a study found that the average pH of effluents from blue crab processing industries was 7.63, with a standard deviation of 0.54; for non-Alaska bottom fish, it was about 6.89 with a standard deviation of 0.69 (Carawan et al., 1979). The pH levels generally reflect the decomposition of proteinaceous matter and emission of ammonia compounds (Gonzalez 1996). pH is significantly low in shrimp pre-processing units compared to other units ($p<0.001$). However, there was no significant variation between months ($p>0.05$) (Table 3.3). Extremes of pH of wastewater are generally not acceptable as extremes of pH may cause problems to survival of aquatic life. It also interferes with the optimum operation of wastewater treatment facilities (Kavitha et al., 2012).

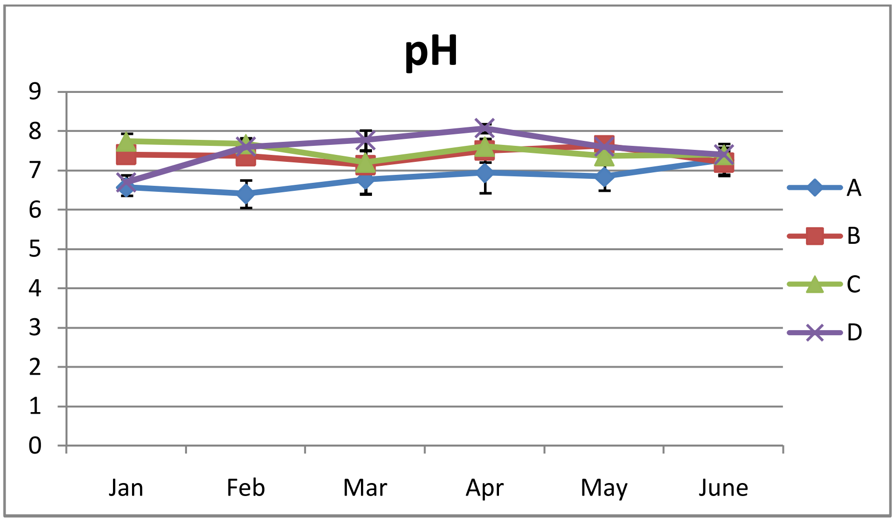

Fig. 3.1: Variation of pH in Prawn (A) and Cephalopod (B) pre-processing, Fish filleting (C) and Prawn processing (D) effluents.

3.9.2 Total Solids

Waste water contains variety of solid materials. Total solids are determined as residue left after evaporation of unfiltered samples. These wastes contribute significantly to the suspended solid concentration of the waste stream. There is a significant variation between different samples studied. TS were significantly higher in shrimp pre-processing centers compared to other centers. ($p<0.01$) and the months of March and April experienced significantly higher value for T S ($p<0.001$) (Table 3.3). The highest value was observed in Sample A, where it varies from1203 mg/L in the month of June to the highest 6754mg/L in April. By contrast the least amount was observed in the processing effluent where it varies from 1800 mg/L to 2905 mg/L. The overall mean value for six months in sample A is 3779.9 mg/L, in sample B is 3035.9 mg/L, in sample C is 2366.6 mg/L and in sample D is 2211.5 mg/L (Fig.3.2).

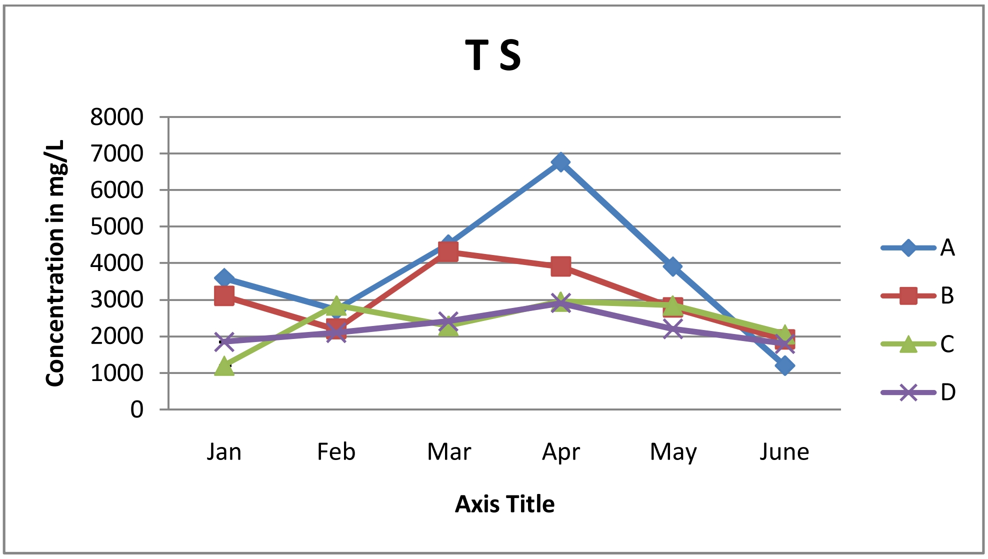

Fig. 3.2: Variation of TS in Prawn (A) and Cephalopod (B) pre-processing, Fish filleting (C) and Prawn processing (D) effluents.

3.9.3 Total Suspended Solids

Total suspended solids play an important role in waste water treatment. TSS test results are routinely used to assess the performance of conventional treatment processes and need for effluent filtration in reuse application. TSS is the samples under suspension and remains in effluent sample. At high concentrations TSS can be aesthetically vexing and upsurge water turbidity, which reduces light availability and photosynthetic activity of algae and others aquatic plants. There is significant difference in TSS between processing units ($p<0.001$), (Table 3.3). In the peeling shed effluent it is significantly higher than all other centers under study ($p<0.001$). In the present study, the mean value in the pre- processing effluent fluctuates between 214 mg/L to 947.6 mg/L, where as in the processing plant effluent it varies from 79 mg/L to 328 mg/L in June. In cuttlefish cleaning effluent it varied from 124.9 mg/L in June to 644 mg/L in March, where as it is comparatively low in fish filleting effluent. The overall mean value for six months in sample A is 680.8 mg/L, in sample B is 355.9 mg/L, in sample C is 125.6 mg/L and in sample D is 191.5 mg/L (Fig. 3.3).

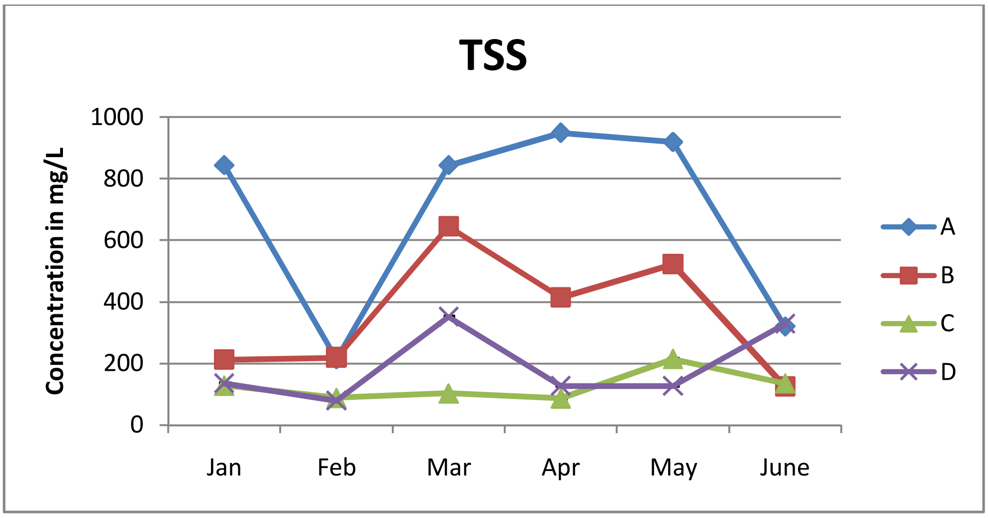

Fig. 3.3: Variation of TSS in Prawn (A) and Cephalopod (B) pre-processing, Fish filleting (C) and Prawn processing (D) effluents.

3.9.4 Ammoniacal Nitrogen (NH_3-N)

Sometimes high ammonia concentration is observed due to high blood and slime content in wastewater streams. The overall ammonia concentration is ranged from 0.7 mg/L to 69.7 mg/L (FREMP 1993). The degree of ammonia toxicity depends primarily on the total ammonia concentration and pH. It is the unionized ammonia is most toxic to aquatic organisms as it can readily diffuse through cell membranes and is highly soluble in lipids (Chin and Chen 1987) The NH_3-N value was too high in all the samples analyzed in the month of March, to other months with 57.8 mg/L (Sample A), 43.6 mg/L (Sample B), 36.2 mg/L (Sample C) and 52.5 mg/L in (Sample D) respectively (Fig. 3.4). There is no significant difference between centers ($p>0.05$), where as statistically significant difference noticed between months, especially in the months of March and April ($p<0.001$) (Table 3.3). However, it is predominantly high in all the months in sample A and sample D. The overall mean value for six months in sample A is 36.1 mg/L, in sample B is 32.6 mg/L, in sample C is 29.1 mg/L and in sample D is 36.2 mg/L.

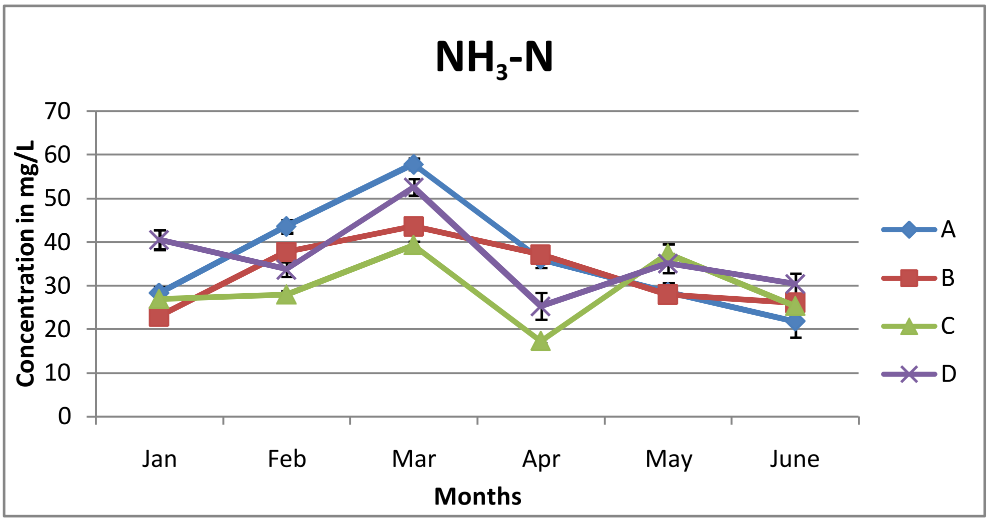

Fig. 3.4: Variation of NH_3-N in Prawn (A) and Cephalopod (B) pre-processing, Fish filleting (C) and Prawn processing (D) effluents.

3.9.5 Oil and Grease (O & G)

No significant difference can be detected in between processing centers and even in months no significant variation was noted ($p>0.05$) (Table 3.3). The highest value observed is 12.25 mg/L in sample C, where it varied from 2.1 mg/L to 12.25 mg/L, at the same time in sample A the value varied between 1.6 to 6.8mg/L, and in sample B it varied from 1.7 mg/L to 3.7 mg/L. however it is considerably less in sample D, where it varied from 0.06 to 1.92 mg/L. The mean value together for all the six months, in sample A is 4mg/L, sample B is 2.9 mg/L, sample C is 5.1 mg/L, and in sample D is 0.9 mg/L (Fig. 3.5)

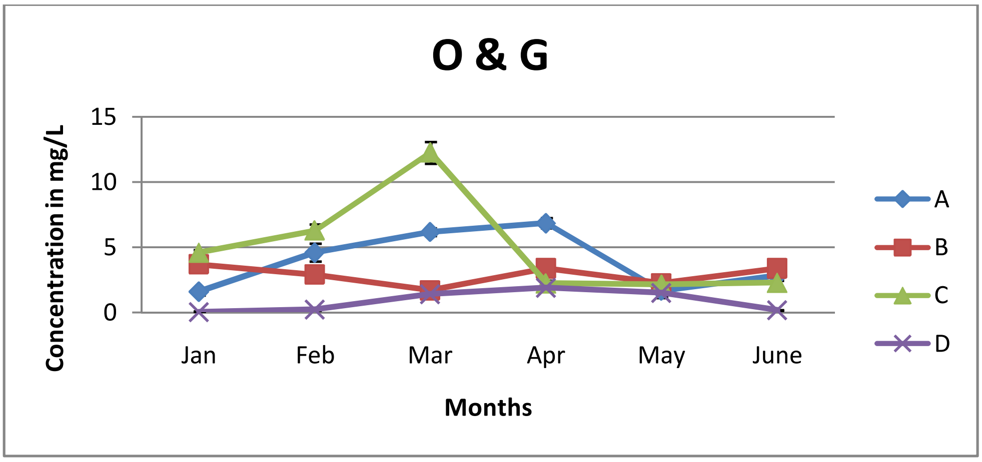

Fig. 3.5: Variation of O & G in Prawn (A) and Cephalopod (B) pre-processing, Fish filleting (C) and Prawn processing (D) effluents

3.9.6 Biological Oxygen Demand (BOD_5)

Biochemical oxygen demand (BOD_5) estimates the degree of contamination by measuring the oxygen required for oxidation of organic matter by aerobic metabolism of the microbial flora. It is also taken as a measure of the concentration of organic matter present in any water. BOD is the most reliable parameter for judging the extent of pollution in the water (Mishra and Saksena 1991). The greater the decomposable matter present, the greater the oxygen demand and the greater the BOD values (Ademoroti, 1996). In seafood-processing effluent biochemical oxygen demand originates from the carbonaceous compounds which are used by microorganisms as their substrate and from the nitrogenous compounds such as proteins and volatile amines. Wastewaters from seafood-processing operations can be very high in BOD_5 (Lawrence et al., 2005). BOD_5 values of sample D is fairly low compared with the sample A. In sample D it varied from 560 mg/L to 1226.6 mg/L, whereas in sample A it ranges from 1266 mg/L to 3600 mg/L. In sample B, it varies from 1466 mg/L to 3166.6 mg/L, while in sample C it ranges from 920 mg/L to 1635 mg/L. Overall mean value for six months together in sample A is 2250 mg/l, sample B is 2061 mg/l, in sample C is 1342 mg/L and in sample D is 964 mg/L. BOD is significantly high in shrimp preprocessing effluent ($p<0.001$). This high BOD may be due to less water consumption by the peeling shed (Uttamangkabovorn et al., 2005). In the months of March and April significantly higher values for BOD ($p<0.001$) (Table 3.3) was experienced. Low value of BOD may be due to lesser quantity of total solids, suspended solids in water as well as to the quantitative number of microbial population (Avasan and Rao, 2001).

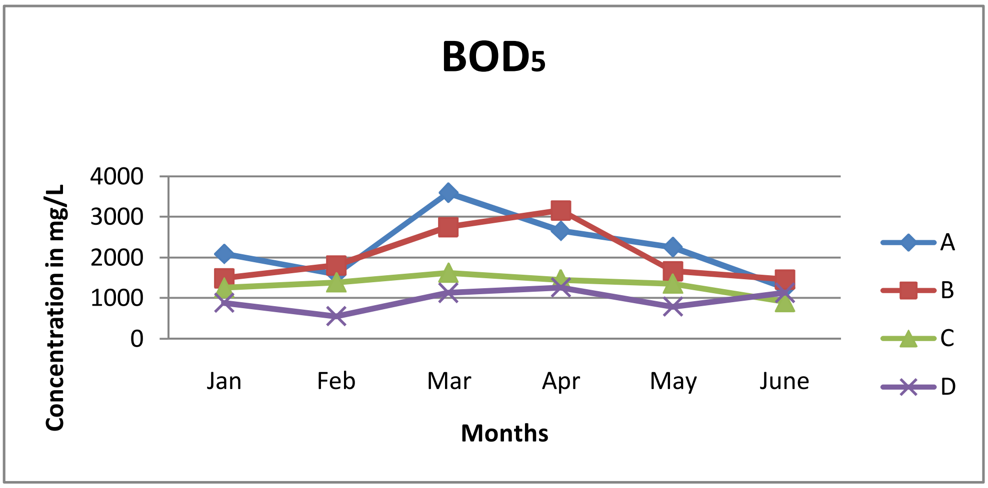

Fig. 3.6: Variation of BOD_5 in Prawn (A) and Cephalopod (B) pre-processing, Fish filleting (C) and Prawn processing (D) effluents

3.9.7 Chemical Oxygen Demand (COD)

The COD is a test which is used to measure pollution of domestic and industrial waste. The waste is measured by the amount of oxygen required for oxidation of organic matter to produce CO_2 and water. COD of an effluent is usually higher than the BOD_5, because the number of compounds that can be chemically oxidized is greater than those that can be degraded biologically. Similar results have been observed by many workers (Ferjani et al., 2000, Mishra and Saksena 1991, Molina 2003). Due to rapid change in salinity soluble COD was increased by the release of cellular material (Kolhe et al., 2008) In the present study COD in sample A ranged from 1666 mg/L to 3666 mg/L, by contrast in sample D it varies from 800 mg/L to 1666 mg/L. In sample B it varies from 2066 mg/L to 3164 mg/L, and in sample C it varies from 1500 mg/L to 2660 mg/L. The overall mean value for six months in sample A is 2700 mg/L, in sample B is 2570 mg/L, in sample C is 1882 mg/l, and in sample D is 1442 mg/L (Fig. 3.7).

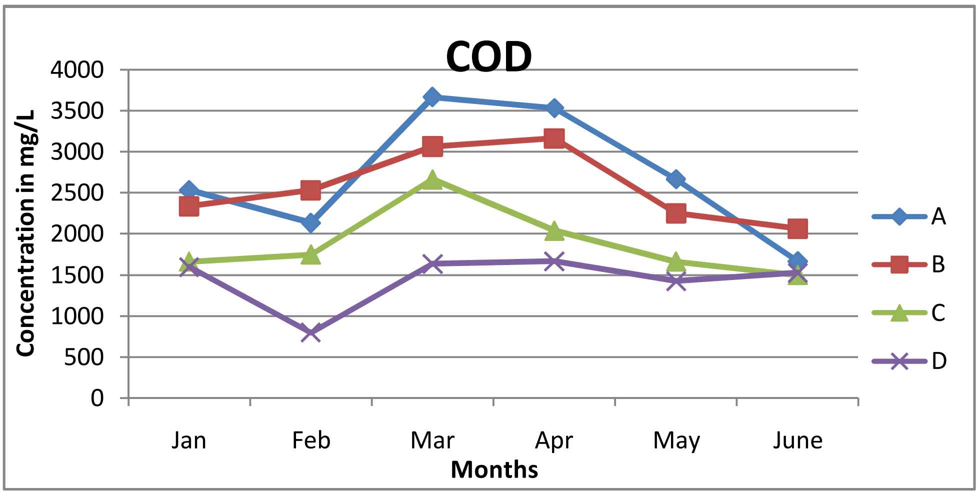

Fig. 3.7: Variation of COD in Prawn (A) and Cephalopod (B) pre-processing, Fish filleting (C) and Prawn processing (D) effluents /

Shrimp preprocessing centers and cuttlefish, squid and octopus processing centers showed significantly higher COD ($p<0.001$). Months of March and April experienced significantly higher value of COD ($p<0.001$) (Table 3.3).

3.9.8 Chloride

Chloride content is more in fish processing effluent, as salt is used for preservation and the highest mean value observed in sample C is 1010 mg/L, while it is predominantly high in sample D in all the months analyzed. The summative mean value for six months in sample A is 800 mg/L, in sample B is 809.7 mg/L, in sample C is 838 mg/L, and in sample D is 875 mg/L (Fig.3.8). No significant difference in Cl could be detected between centers ($p>0.05$). Between month's variation were not significant in the case of chloride ($p>0.05$) (Table 3.3).

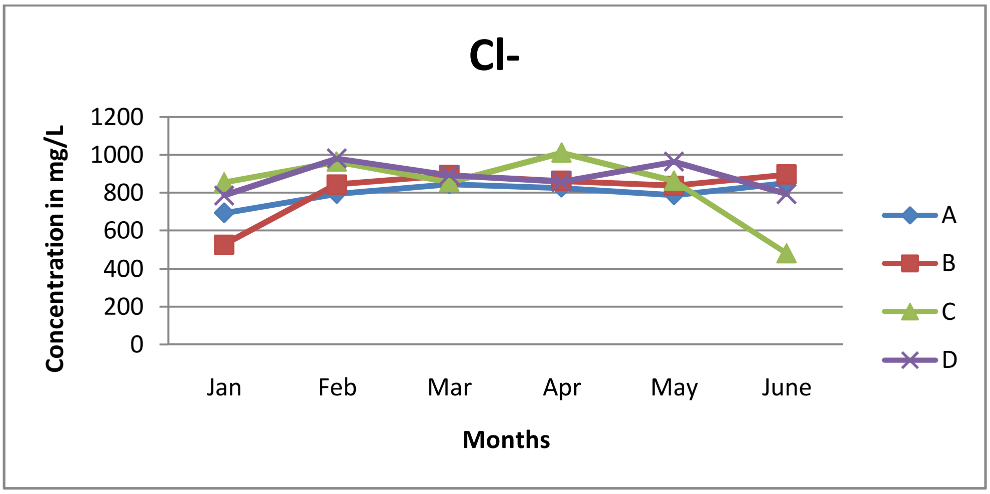

Fig. 3.8: Variation of Cl- in Prawn (A) and Cephalopod (B) pre-processing, Fish filleting (C) and Prawn processing (D) effluents

3.9.9 Alkalinity

Alkalinity of water is its acid-neutralizing capacity. It is the sum of all the titrable bases. The measured value may vary significantly with the end point pH used. Alkalinity measurements are used in the interpretation and control of water and wastewater treatment processes. The organic matter rich processing waste was responsible for increase in the carbonate and bicarbonate values which lead to shift in alkalinity values Seenaya (1971). According to that the highest value recorded was in sample B with a figure of 796 mg/L and the least was in sample A 324 mg/L. The mean value together for six months in sample A is 410 mg/L, in sample B is 555.3 mg/L, in sample C is 486 mg/L and in sample D is 505.5 mg/L (Fig.3.9). Alkalinity is significantly higher in cuttlefish, squid and octopus processing centres and shrimp processing effluent ($p<0.01$), where as month's variations were not significant ($p>0.05$) (Table 3.3).

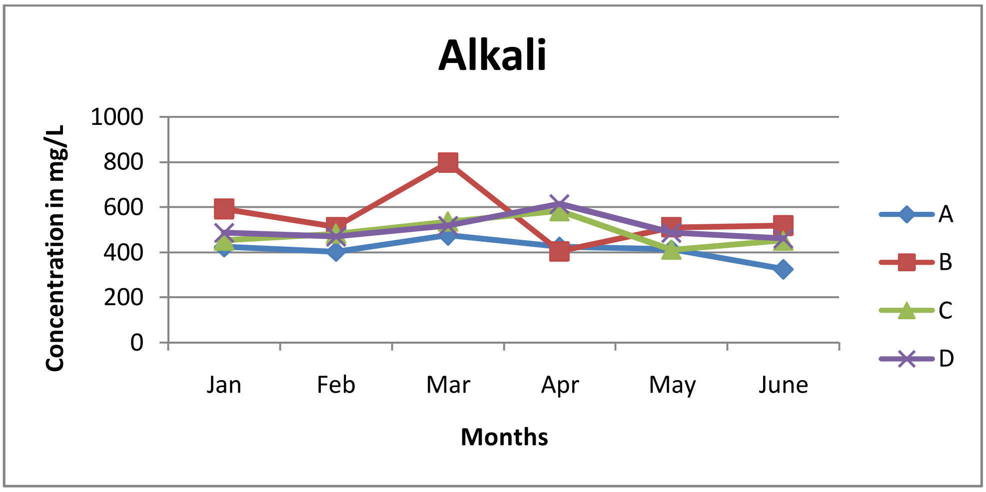

Fig. 3.9: Variation of Alkali in Prawn (A) and Cephalopod (B) pre-processing, Fish filleting (C) and Prawn processing (D) effluents

3.10 Heavy Metal Analysis by ICP AES System

Cd and Hg were BDL in all the samples from peeling (shrimp) effluent. Pb was present in 0.01 ppm to 0.06 ppm in four out of six samples. In sample B, Cephalopod processing effluent Cd was detected in four out of six samples. (0.01 to 0.05 ppm). Pb was present in all the samples except one (0.01 to 0.1 ppm), Mercury was BDL. In sample C, fish filleting effluent Cd and Hg were absent, where as Pb was present in five samples (0.1 to 0.6 ppm). In shrimp processing effluent, sample D, Cd and Pb were detected and Hg below detectable level. Cd was present in 4 samples (0.1 to 0.5 ppm), and Pb was in 5 samples (0.1 to 0.6 ppm) (Table 3.4)

Hg was below detectable level in all the samples analyzed, whereas Pb was present in almost all the samples tested. Presence of Pb and Cd in the processing effluent may be from the contamination of natural habitat of the organism, or from the polluted ground water that is used for washing. Fish harvested from waters that have been exposed to processing wastes have higher probability to accumulate toxic compounds in their tissues. (FDA. 2001 and Lopes, 2004)

Cadmium is a common water and sediment pollutant in waters near to industrial facilities, and is a cumulative poison and a maximum level of 0.05 ppm is permitted for drinking water. Lead is the most abundant heavy metal occurring in nature and is used by man on a large scale. Mostly Pb is received from food, other sources such water in areas with Pb piping and plumb solvent water, air near point source emissions, soil dust and paint flakes.

Table 3.4. Heavy metal analysis by ICP AES system

Sample	Months	Cd2144	Pb2203	Hg1849	Unit
Shrimp Preprocessing (sample A)	Jan.	BDL	BDL	BDL	Ppm
	Feb.	BDL	0.01	BDL	Ppm
	Mar.	BDL	0.06	BDL	Ppm
	Apr.	BDL	0.02	BDL	Ppm
	May	BDL	0.04	BDL	Ppm
	Jun.	BDL	BDL	BDL	Ppm
Cephalopod processing (Sample B)	Jan.	0.01	0.06	BDL	Ppm
	Feb.	0.01	0.04	BDL	Ppm
	Mar.	BDL	0.01	BDL	Ppm
	Apr.	0.05	0.10	BDL	Ppm
	May	BDL	0.06	BDL	Ppm
	Jun.	0.04	BDL	BDL	Ppm
Fish Filleting (Sample C)	Jan.	BDL	0.01	BDL	Ppm
	Feb.	BDL	0.1	BDL	Ppm
	Mar.	BDL	BDL	BDL	Ppm
	Apr.	BDL	0.01	BDL	Ppm
	May	BDL	0.06	BDL	Ppm
	Jun.	BDL	0.01	BDL	Ppm
Shrimp Processing (Sample D)	Jan.	0.01	0.01	BDL	Ppm
	Feb.	0.02	0.01	BDL	Ppm
	Mar.	BDL	0.06	BDL	Ppm
	Apr.	0.02	0.01	BDL	Ppm
	May	BDL	0.04	BDL	Ppm
	Jun.	0.005	BDL	BDL	Ppm
Detection Limit		0.01	0.03	0.05	Ppm

*BDL-Below Detection Limit

3.11 Microbiological Analysis Data

From the microbiological study, it is noted that, fecal coliform and *E. coli* concentrations dominated in the effluent of Seafood Processing unit (Shrimp peeling effluent, Cephalopod processing effluent, Fish filleting effluent and Shrimp processing effluent) (Table 3.5). Maximum number of faecal coliforms were detected in sample (A) Shrimp peeling effluent followed by shrimp processing effluent sample (D), where as the least number was observed in the fish filleting effluent. Shrimp peeling effluents contain more organic as well as inorganic matters and in turn a greater number of bacteria. Bacteria can arise from the discharge of untreated sewage that passes into rivers, lakes and coastal waters. Findings from Rashid et al. (1992) are in agreement with the statement. According to them, the bacterial load on shrimps increased along the different steps in processing mainly due to contamination from ice, water, contact surface of utensils and the workers hands. These microbes have public health relevance and concern. It is possible that fish or shellfish which consume discharged effluent may be colonized by these pathogens and cause food borne diseases associated with seafood consumption (Ward 1989; Price 1995). A noticeable change could be observed between two seasons, pre-monsoon and monsoon. In pre-monsoon the MPN number of microbial flora was very high in all the samples compared to that of rainy season. This can be attributed to the highest production rate during the months of March, April and May (pre-monsoon). When there is more production less water will be consumed. In monsoon, because of trawling ban, seafood processing is less compared to that of pre monsoon. Desirable limit of faecal coliform is 1000 MPN/100ml. and a maximum permissible limit 10,000 MPN/100ml (CPCB standards).

Table 3.5: MPN/100 ml for two seasons (Pre monsoon and Monsoon) in shrimp and cuttlefish preprocessing, fish filleting and shrimp processing effluents.

		Growth medium	Preprocessing (peeling)	Cuttlefish	Fish filleting	Shrimp processing
PM	March	L B	1100+	210	75	43
		BGLB	1100+	210	150	150
		EC	1100+	43	75	43
		EMB	1100+	43	75	43
	April	L B	150	28	75	75
		BGLB	150	75	150	93
		EC	43	43	75	75
		EMB	43	93	75	75
	May	L B	1100	75	20	20
		BGLB	150	150	150	93
		EC	1100	150	20	75
		EMB	460	150	20	75
M	June	L B	1100+	28	35	35
		BGLB	210	150	20	93
		EC	210	150	75	75
		EMB	210	150	75	75
	July	L B	1100+	35	28	120
		BGLB	1100+	20	150	150
		EC	1100+	28	75	75
		EMB	1100+	28	75	75
	AUG	L B	1100+	35	20	210
		BGLB	150	28	20	93
		EC	120	28	15	75
		EMB	120	28	15	75

3.12 Summary and Conclusion

Analyzed samples showed that pH ranged 6.8 to 7.5 in all the samples studied and in TS there was significant variation between samples studied. Lowest was observed in processing effluent where it ranges from 1800 to 2905mg/L and the highest value was noted in the shrimp preprocessing effluent 1203 to 6754 mg/L. Significant variation was observed between processing centers and a maximum was observed in shrimp peeling effluent. NH_3-N value was too high in sample A (shrimp peeling) 57.8 mg/L. Oil and Grease was much more in fish filleting effluent compared with other samples. A high BOD value was observed in shrimp peeling effluent where it ranges from 1266 mg/L to 3600 mg/L and a low BOD was noted in sample D the shrimp processing effluent and varies from 560 mg/L to 1266 mg/L. A high COD ranged from 1666 mg/L to 3666 mg/L was observed in the shrimp peeling effluent by contrast, COD in shrimp processing effluent was with 800 mg/L to 1666 mg/L. However, fish filleting effluent was noted for its high chloride content with 1010mg/L and cephalopod processing effluent was noted for its high alkalinity. Heavy metals such as Cd and Pb were present in most of the samples analyzed. In addition to that presence of fecal coliform and *E.coli* were detected in all the samples of which shrimp preprocessing effluent was dominant.

Effluents from seafood industries are heavily loaded with organic as well as inorganic contaminants that are indiscriminately discharged into water bodies will have profound impacts on ecosystem. Continuous flow of effluents from large number of industries would reduce the assimilative capacity of the water bodies and there by damaging its self purification system by nutrient enrichment in the coastal and inland waters. These effluents have the potential to generate chronic toxicity problems on fish habitat and marine environmental quality. In addition to the sedimentary habitat problem, the effluent could also lead to contaminant build up in sediments, resulting in marine acute toxic contamination of fish and shell fish species of commercial importance.

Hence proper treatment of seafood processing effluents before discharging into water bodies is strongly suggested to protect the aquatic ecosystem.

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Chapter 4

ASSESSMENT OF THE EFFICACY OF THE EXISTING TREATMENT SYSTEM

Contents

4.1 Introduction

Wastewater from seafood processing plants can be characterized by high concentration of nutrients, high levels of nitrogen content found as ammonia (NH_3-N; 29 to 35 mg-L-1), high total suspended solids (0.26 to 125,000 mg-L-1), increased BOD (10 to 110,000 mg –L-1), and COD (496 to 140,000 mg-L-1), and by the presence of cleansers (AMEC Earth and Environmental Limited 2003).

In the last 40 years various drastic ecological changes and human disasters have arisen, majority of which are from industrial wastes causing environmental degradation (Abdel-Shafy and Abdel- Basir, 1991; Sridhar et al., 2000). The effluents from these industries are bio hazardous to all living organisms in the environment because they contain toxic substances detrimental to health (Adebisi et al., 2007; Adriano, 2001; Bakare et al., 2003). The most polluted fraction is the waste water derived from the fish preservation. Recently, there has been an alarming and distressing increase in organic pollutants (Nadal et al., 2004). As many effluents are not treated properly, these products are discharged on the ground or in the water bodies and most of these discharges accumulate in the system through food chain (Odiete, 1999). The waste water discharge form industries are the major source of pollution to the ecosystem (Morrison et al., 2001). The degradation of environment is a result of the adverse effect of industrial waste on living organism and agriculture (Anikwe and Nwobodo, 2006). There are several techniques used for treatment of wastewater from fish and surimi industries (Wu.et al., 2002).

Seafood processing facility uses large amount of water for various purposes and in turn generate a high volume of wastewater from a variety sources such as fish unloading, dressing, equipment spraying, process additives, equipment disinfection and facility cleaning. Water is not only used for fish cleaning but also flesh offal and blood from equipment and floors and also flume the offal to floor drains and collection sumps. The processing equipment sometimes has inbuilt water sprays to keep the equipment clean and to flush offal away. Other than resulting in high water consumption this method of equipment cleaning and offal transport causes the mixing of the rinse water with offal and blood, which has two main disadvantages.

- When the blood which contains soluble BOD mixes with water, it cannot be removed by physical treatment such as screening.
- The rough wastewater pumping action on offal chunks resulting in an increased bulk of smaller particles and that may pass through the flowing screen. In addition to this the pumping action is supposed to increase the dissolved BOD content by solubilizing suspended organic material. Hence the dissolved compounds are discharged unchanged as they cannot be removed by physical treatment such as screening.

Fish processing plants generate large wastewater volume and are frequently inefficient users of water (World Bank Group, 2013). Reports show that pollution control in seafood processing plants in Thailand could be achieved through water conservation and wastewater reuse (Achour et al., 2000). The main environmental problems of these industries are high water consumption and presence of high organic matter, oil and grease, ammonia and salt. Biological treatments of this wastewater make them harmless. At all times when food in any form is handled, processed, packaged and stored, there will be an inherent generation of waste water. The quantity and general quality (pollutant concentration and composition) of processing waste water has both environmental and economic consequences with respect to its treatability and disposal. The economics of waste water lie in the amount of product loss from the processing operations and the cost of treating the waste materials. The cost for product loss is very clear, however, the cost for treating the wastewater lies in its specific characteristics. Two significant characteristics which control the cost for treatment are the daily volume of discharge and the relative strength of the wastewater. Other important characteristics that affect system operations and specific discharge limits or restrictions are identified.

Excessive discharge of organic nutrients into the marine environment can result in reduction of dissolved oxygen in the water, leading to hypoxia or anoxia, increased ammonia concentrations, overloads of nitrogen (N) and phosphate (P) which in turn leads to excessive plant growth, variation in pH and increased water turbidity (Tchoukanova et al., 2003)

Numerous studies have been shown that wastewater from some seafood processing plants fail toxicity tests such as the 96 hour acute lethality test on three spine stickle back (*Gasterosteus aculeatus*) and rainbow trout (*Onchorhynchus mykiss*), and microtox assays. These suggest possible acute and chronic toxicity problems for fish living in the receiving environment. (Wells and Shneider, 1975; Nova Tec Consultants Inc. and EVS, 1994)

The environmental consequences in not adequately removing the pollutants from waste stream can have serious ecological impacts. For example, if inadequately treated wastewater were to be flown to a stream or river, an eutrophic condition would develop within the aquatic environment as a result of the discharge of biodegradable, oxygen consuming compounds. If this condition sustained for a sufficient amount of time, the ecological balance of the receiving stream, river or lake (i.e., aquatic micro flora, plants and animals) would be upset. Continual depletion of the oxygen into these water systems would also result in the development of obnoxious odors and unsightly scenes.

4.2 Wastewater Treatment

Wastewater treatment options for fish processing plants can be divided into physical, chemical and biological treatment (Fig. 4.1). Depending on the degree to which organic materials are collected separately or mixed into the effluent stream there is a fusion of primary and secondary treatment facilities are practiced in the seafood processing facilities.

Waste water deriving from industries has to be pre- treated by the industry before being released to a waste water treatment plant. Different pre-treatment steps, as well as post treatment steps, can be added in order to improve the treatment for industrial waste water (Subramanian et al., 2010).

Physical treatment options make use of differences in physical properties between water and contaminants for their separation. Chemical treatment is generally required to improve removal efficiencies. With the exception of ultra filtration, physical treatment methods cannot remove BOD which is associated with dissolved substances. This fraction of the overall BOD can be substantial and can only be removed by chemical and /biological treatment.

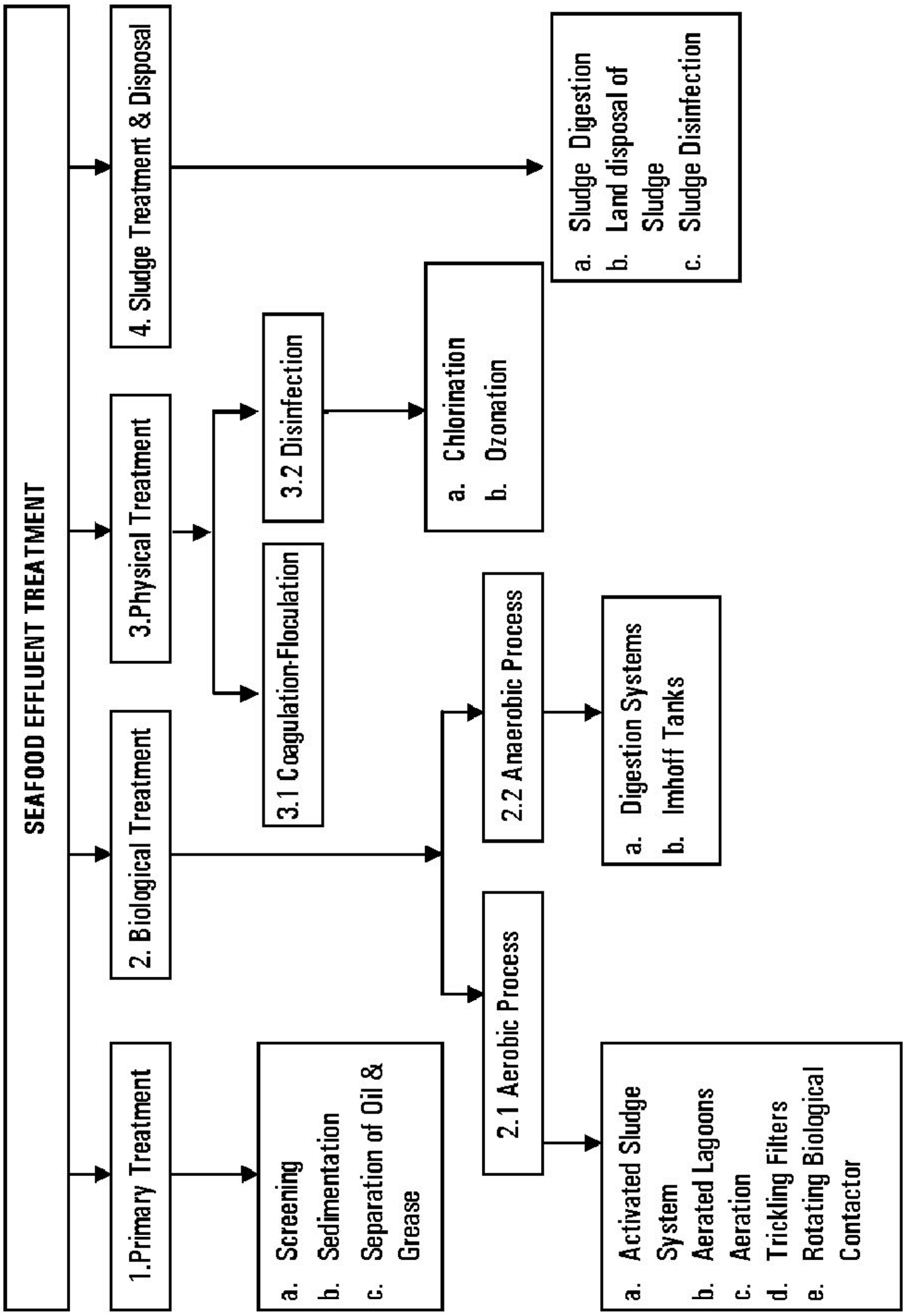
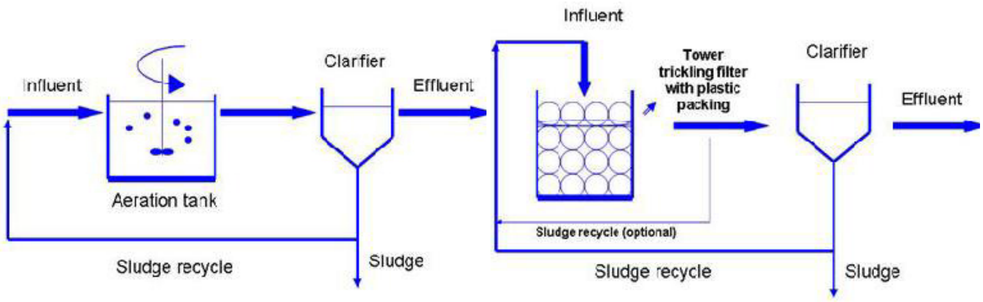


Fig. 4.1: Seafood effluent treatment methods classification, adapted from Genovese and Gonzalez, 1998

4.2.1 Biological treatment



(a) (b)

Fig. 4.2: Flow diagram of a typical (a) activated sludge system and (b) trickling filter system. Adapted from Regina, (2009); modified from Metcalf and Eddie, (2003).

After suitable primary treatment the wastewater is treated through a biological wastewater treatment system where microorganisms are involved in degradation of organic matter, consists of anaerobic and aerobic treatment system. It is an advantage for the biological treatment if the wastewater coming into the system is homogenous (Persson, 2005). The process is complex and not dependable as it is not stable (Bing- Jie and Yu Han, 2011), therefore the obtained reduction can be unpredictable and vary from one time to another.

Anaerobic processes such as up flow anaerobic sludge blanket (UASB) reactor, anaerobic filter (AF) and anaerobic fluidized bed (AFB) reactor can achieve high (80-90%) organics removal and produce biogas. Aerobic processes such as activated sludge, rotating biological contactor, trickling filter and lagoons are also suitable for organics removal.

Table.4.1: Performance of aerobic and anaerobic systems for fish processing wastewater treatment, adapted from Chowdhury et al. 2010

Process	Fish processing Industry	Raw wastewater characteristics	Organic loading	Organic Removal	Remarks	Reference
Aerobic Activated Sludge	Fish Processing Industry		0.5kg BOD_5/m^3d	90-95% BOD_5	Detention time 1-2d; F/M0.1-0.3 Sludge age 18-20 days	Carawan et al; (1979)
Rotating biological contactor	Fish cannery	pH 6–7; COD 6000–9000; BOD 5100; TSS 2000; TKN 750	0.018–0.037 kg COD/m2 d	85–98% COD	HRT 48 d; effluent TSS 290 mg/L	Najafpour et al. (2006)
Trickling filter	Squid processing	BOD 2–3000	0.08–0.4 kg BOD5/ m3 d	80–87% BOD5		Park et al. (2001)
Aerated lagoon	Fish-processing Industry			90–95% BOD5	Retention time 2–10 d; ponds 2.4–4.6 m deep	Carawan et al. (1979)
Anaerobic Anaerobic fluidized bed reactor Anaerobic fixed filter	Fish cannery (Herring brine)	COD 90000; BOD 78000; oil/fat 4000; total N 3000; SS 10,000; pH 3.8	6.7 kg COD/m3 d 4.7 kg COD/m3 d	88% COD 85% COD		Balslev-Olesen et al. (1990)
Anaerobic filter	Seafood processing Seafood Processing Tuna condensate	Volatile acids 3340	0.3–0.99 kg COD/m3 d 1.67 kg COD/m3 d	78–84% COD 60% COD	HRT 36 days OLR 2.0 kg COD/ m3 d initiated system failure	Prasertsan et al. (1994)
Anaerobic digester	Tuna cooking Mussel cooking	COD 34,500; TS 4000; Cl_ 14 g/L COD 18,500TS 1400Cl- 13 g/L	4.5 kg COD/m3 d 4.2 kg COD/m3 d	80% COD 75–85% COD	HRT 5 d	Mendez et al. (1992)
Anaerobic fixed film	Tuna processing Industry		2 kg COD/m3 d	75% COD		Veiga et al. (1991)
Upflow anaerobic sludge blanket reactor	Mixed sardine and tuna canning	COD 2718 ± 532; lipids 232 ± 29; TKN 410 ± 89; pH 7.2–7.6	1–8 kg COD/m3 d	80–95% COD	HRT 7.2 ± 2.8 h, 61 ± 17%/COD conversion to methane	Palenzuela-Rollon et al. (2002)
Integrated bioprocess Physical pretreatment + anaerobic digester + activated sludge bioreactor	Tuna processing	pH 6.96; TSS 1575; COD 5553; BOD 3300; TKN 440; f at 1450	1.2 kg COD/m3 d	85–95% COD		Achour et al. (2000)

a Values in mg/L except pH.

Land consideration alone can be an inhibitive factor in applying biological methods for pollution slacken in coastal zones (Chao et al.,1980). Most of the seafood operations are seasonal as they are dependent on the availability of the raw materials. The seasonal nature and intermittent processing of the industry makes almost any biological system, except lagoons, impossible to use (Riddle and Shikaze, 1973). However different types of biological treatment are successfully applied in seafood processing industries. In general biological treatment may be used to reduce toxicity caused by high ammonia concentrations and /or BOD levels.

Table 4.2: Table summarizes those factors when applied to aerobic treatment process

(A) Operating characteristics			
System	**Resistance to shock loads of organics or toxics**	**Sensitivity to intermittent operations**	**Degree of skill Needed**
Lagoons	*Maximum*	Minimum	Minimum
Trickling filters	Moderate	Moderate	Moderate
Activated	Minimum	Maximum	Maximum
(B) Cost considerations			
System	Land needed	Initial costs	Operating costs
Lagoons	Maximum	Minimum	Minimum
Trickling filters	Moderate	Moderate	Moderate
Activated	Minimum	Maximum	Maximum

Adapted from Rich 1980

Table 4.3: Industry specific effluent standards for seafood processing industries and general standards, Source CPCB

Parameter	Seafood industry specific standards	General standards (Not to exceed, limit in mg/L)			
		Inland surface water	Public sewer	Land for irrigation	Marine coastal areas
BOD (3 days at 27°C)	<30mg/L	30	350	100	100
TSS	<50mg/L	100	600	200	a.100 for process wastewater b. 10% above of the influent cooling water for cooling water effluent
Oil & Grease	<10mg/L	10	20	10	20

4.3 Wastewater treatment methods adopted by the plants in the study area

Seafood processing effluent treatment is found to be a complex and costly process. High strength wastewaters and highly variable seasonal loadings make many treatment methods ineffective and not cost efficient.

Three major types of wastewater treatment methods which are prevalent in the area studied. They are:

i). Conventional Septic Tank System (CSTS)

ii). Aerobic Treatment System (ATS)

iii). Nano Dispersion and Flocculation System (NDFS)

4.3.1 Conventional Septic Tank System (CSTS)

It is similar to our domestic septic tank concept. Here also the bacterial process is carried out in the absence of oxygen (anaerobic). The temporary holding of wastewater in a covered tank, where heavy solids can settle to the bottom while lighter solids along with oil and grease float on the surface, comprises the primary treatment. Wastewater enters the first chamber where the solids get settled and undergoes anaerobic digestion. The scum float on the surface is removed and the remaining liquid is discharged directly without any treatment. In certain cases wastewater just passes through the sedimentation tank without providing sufficient time to settle down the solid particles. In majority of the cases the capacity of the tanks are not enough to hold the entire wastewater generated per day. An important feature of anaerobic digestion is the production of biogas. Since there is no aeration in the tanks practically there is no power consumption. Often large areas are required for the treatment.

4.3.2 Aerobic Treatment System (ATS)

A pretreatment of wastewater takes place in an aerobic treatment system, by adding air to breakdown organic matter, reduce pathogens, and transform nutrients. Compared to conventional septic tank system, ATS breaks down organic matter more efficiently and reduce the concentration of pathogens in the effluent. By bubbling compressed air through liquid effluent in a tank, ATSs create a highly oxygenated (aerobic) environment for bacteria, which uses the organic matter as an energy source. In the next stage bacteria and solids settle out and the cleaner effluent is discharged directly. ATS usually have a bar screen chamber ahead of collection tank that removes the large solids and provides protection. ATS consists of a collection tank, aeration tank, secondary settling tank (hopper bottom settling tank) followed by a filter feed cum disinfection tank, pressure sand filter and activated carbon filter and outlets. The sludge from settling tank is pumped to sludge drying bed (30 %) and the balance is pumped back aeration tank. The bacteria used for decomposition are grown on a specific surface medium and air is provided to that part of the tank. Decomposition of organic matter limited to this area and settling occurs in a second chamber. Even though this design is expensive, the effluent is of high quality if it is properly maintained.

4.3.3 Nano Dispersion and Flocculation System (NDFS)

If there is more number of seafood processing plants in one area, a common effluent treatment plant is beneficial as it greatly reduces the general overhead and space requirements when compared to independent units. There is a common effluent treatment plant installed in Aroor industrial area under the guidance of Central Institute of Fisheries Technology (CIFT). Here a Nano Dispersion and Flocculation System is used to treat the effluents. More literature is not available.

However all these methods are available, the most prevalent treatment method followed in the study area is activated sludge treatment system. Majority of the preprocessing plants use only the physical methods. Even if all the treatment facilities are present they are not running it properly due to high electricity charge and maintenance cost.

Objective of this study is to evaluate the efficacy of the existing treatment system by analyzing the physicochemical characters of the effluent before and after treatment, and to assess to what extent the present system is effective.

4.4 Materials and methods

Untreated wastewater and treated wastewater were collected from three seafood processing facilities, where three types of effluent treatment methods are followed such as Nano Dispersion and Flocculation System (NDFS), Aerobic Treatment System (ATS) and Conventional Septic tank System (CST). Samples were collected monthly once for four months, characterization of the wastewater evaluated in terms of pH, TSS, TS, BOD, COD, NH_3, NO_2-N, NO_3-N and O&G for the influent and effluent (untreated and treated) by following APHA protocols and the quality of the treated water was compared with general effluent standard set by Central Pollution Control Board (CPCB). The statistical significance of the data collected were analyzed using student's ‘t’ test by comparing means of two samples, untreated and treated. Those parameters which show significant change in treated wastewater were selected for comparison between device types using ANOVA and Tukey HSD test.

4.5 Results and Discussion

Table 4.4: Physico chemical parameters of untreated and treated wastewater from NDFS, ATS and CST

ETP	Parameter	Treatment Type		Df	T	P Value
		Untreated (Mean)	Treated (Mean)			
NDFS	pH	6.560	6.958	5.584	-2.4104	p>0.05
	TSS	46.22	24.22	4.425	1.8629	p>0.05
	TS	1141.8	165.8	4.555	40.2742	P<0.001*
	BOD	917.6	22.5	4.001	4.483	P<0.001*
	COD	1408	211.8	4.009	4.9733	P<0.001*
	O&G	1.086	0.150	4.131	2.4061	p>0.05
	NH_3-N	41.87	10.88	5.973	5.5241	P<0.001*
	NO_2-N	2.29	2.22	7.893	0.0981	p>0.05
	NO_3-N	0.1744	5.5100	4.048	-2.651	P<0.05*
ATS	pH	7.22	7.07	5.869	0.428	p>0.05
	TSS	217.568	46.620	4.251	7.9046	P<0.01*
	TS	1695.40	461.38	4.614	13.9455	P<0.001*
	BOD	1361.2	116.3	4.064	14.8029	P<0.001*
	COD	1756.6	234.2	4.457	21.7202	P<0.001*
	O&G	1.706	0.320	4.601	2.8996	P<0.05*
	NH_3-N	44.832	17.388	7.814	3.8288	p>0.01*
	NO_2-N	3.484	4.372	4.359	-0.7919	p>0.05
	NO_3-N	0.1784	1.9040	4.574	-2.8784	P<0.05*
CST	pH	7.140	7.016	6.15	0.4009	p>0.05
	TSS	202.40	81.92	5.168	3.8683	P<0.01*
	TS	1905.6	1241.2	6.219	1.9266	p>0.05
	BOD	984	516	5.524	3.9966	P<0.01*
	COD	1455.2	916.0	6.51	3.5266	P<0.01*
	O&G	1.758	0.488	5.177	3.6336	P<0.05*
	NH_3-N	50.232	55.630	7.27	-1.3158	p>0.05
	NO_2-N	6.52	23.22	7.053	-4.9752	P<0.05*
	NO_3-N	0.1738	1.0380	5.071	1.9096	p>0.05

Table 4.5: Comparison of untreated and treated effluent parameters with Environmental standards.

Parameters	Environmental standards	NDFS		ATS		CST	
		Untreated effluent	Treated effluent	Untreated effluent	Treated effluent	Untreated effluent	Treated effluent
pH	5.5-9**	6.56	6.95	7.22	7.07	7.1	7.0
TSS mg/L	<50 *	46.22	24.22	217.5	46.20	202.4	81.92
TS mg/L	<500***	1141.8	165.8	1695.40	461.38	1905.6	1241.2
BOD mg/L	<30*	917.6	22.5	1361.2	116.3	984	516
COD mg/L	<250**	1408	211.8	1756.6	234.2	1455.2	916.0
O&G mg/L	<10*	1.086	0.150	1.706	0.320	1.758	0.488
NH_3-N mg/L	<50**	41.87	10.88	44.832	17.388	50.232	55.630
NO2-N mg/L	<10**	2.29	2.22	3.484	4.372	6.52	23.22
NO3-N mg/L	<20**	0.1744	5.5100	0.1784	1.9040	0.1738	1.0380

(*CPCB fish processing industry standard, ** general effluent standard,***WHO drinking water standard)

4.5.1 pH:

pH of the individual sample was measured immediately after its collection by using a pH meter, results are shown in (Table 4.1). Extremes of pH in the effluents are generally not acceptable as that may affect aquatic life. The pH of the influent was 6.5 to 7.2 and the effluent was about close to neutral. pH showed no significant difference between untreated and treated effluents in NDFS, ATS and CST ($p>0.05$). But the pH value is in the permissible limit of 5.5 to 9 as per the general effluent standard prescribed by Central Pollution Control Board (Table 4.3).

4.5.2 Total Solids (TS)

In NDFS total solids in the untreated wastewater was 1141 mg/L and was reduced to 165.8 mg/L after treatment ($p<0.001$). In ATS before treatment value was 1695.4 mg/L and a significant reduction was observed in the post treatment value ($p<0.001$), (Table 4.4). In both the systems TS reduced significantly, below the industry specific standard set by CPCB (<500 mg/L) (Table 4.3) whereas in CST, no significant reduction noted in the treated (1241.5 mg/L) with that of the untreated (1905.6 mg/L) ($p>0.05$) (Table.4.4) and was not up to the industry specific standard. NDFS and ATS are capable of controlling T S; however, NDFS seemed to be more efficient.

4.5.3 Total Suspended Solids (TSS)

TSS doesn't mean that they are suspended or floating matters which remain on top of water layer, but they are under suspension and remain in water sample. It plays an important role in water and wastewater treatment. Excess TSS causes depletion of oxygen in the water body. In ATS, TSS in the untreated effluent were 217.56 mg/L and in the treated it was 46.620 mg/L, a significant difference ($p<0.01$) between two values. In CST, after value (81.92 mg/L) was comparatively less than the before value (202.40 mg/L) ($p<0.01$) (Table 4.4) Where as in NDFS, compared to ATS and CST, the pre value (46.22 mg/L), and post value (24.22 mg/L) which was almost half of the before value and was not statistically significant ($p>0.05$). But in NDFS and ATS treated effluent values are well within the maximum permissible limits of 50 mg/L set by CPCB standards (Table 4.3).

4.5.4 Biological Oxygen Demand (BOD_5)

In the treated wastewater BOD was significantly reduced in all the three devices. In NDFS, BOD in the untreated effluent was 917.6 mg/L and in the treated it was 22.5 mg/L. There was a sharp decrease in the treated wastewater and is statistically significant ($p<0.001$). In ATS it was 13651 mg/.L in the untreated effluent and in the treated it was 116.3 mg/L and had a significant change ($p<0.001$). Whereas, in CST, BOD value before treatment was 984 mg/L and after treatment it was 516 mg/L ($p<0.01$). (Table. 4.4). BOD in the treated wastewater very seldom came below the industry specific limit prescribed by CPCB (<30 mg/L). Both ATS and CST are not able to keep the BOD level below 30 mg/L. Literature showed that properly maintained aerobic treatment system can clearly reduce the BOD (Table 4.1), but most of the plants are not properly maintaining the treatment facility in order to reduce the operational cost. However, only NDFS met the industry specific standard prescribed by CPCB (<30 mg/L) (Table 4.3).

4.5.5 Chemical Oxygen Demand (COD)

Before the treatment the value of COD in NDFS was 1408mg/L and that of the one after treatment was 211.8 mg/L and showed a significant change where $p<0.001$. In ATS, the value before treatment was 1756.6 mg/L and the after treatment was 234.2 mg/L which is very significant ($p<0.001$). Whereas, in CST the untreated COD was 1455.2 mg/L and after treatment it came down to 916 mg/L ($p<0.01$) (Table 4.4). Treatment efficiency is much higher in both NDFS and ATS where the COD was <250 mg/L, minimum permissible limit of general effluent standard set by CPCB (Table 4.3) compared to conventional septic tank system where COD was >250mg/L.

4.5.6 Oil & Grease (O & G)

After treatment the values were significantly lesser than the one before treatment in aerobic treatment and in conventional septic tank system ($p<0.05$). In ATS, O & G in the untreated wastewater was 1.706 mg/L and in the treated effluent it was 0.320 mg/L and in CST, it was 1.758 mg/L in the untreated and 0.488 mg/L in the treated effluent. Whereas in NDFS the differences before and after the treatment were not significant ($p>0.05$) where O & G was 1.086 mg/L in the untreated and 0.150 mg/L after treatment (Table 4.4). However, the oil and grease contents always below the CPCB fish processing industry standard <10 mg/L for both untreated and treated effluent (Table 4.3).

4.5.7 Ammoniacal Nitrogen (NH_3-N)

Statistically significant difference was observed between untreated and treated effluents of NDFS and ATS. In NDFS, NH_3-N in the untreated effluent was 41.87 mg/L and in treated it was 10.88 mg/L ($p<0.001$). In ATS it was 44.832 mg/L in untreated and 17.388 mg/L in treated and are significant ($p<0.01$). On the other hand in CST there was no significant difference between the untreated and treated effluents, 50.232 mg/L and 55.630 mg/L respectively. There was no significant difference ($p>0.05$), moreover the values in treated effluent increased considerably. However, the initial values were almost below the CPCB general effluent standard, <50 mg/L (Table 4.3).

4.5.8 Nitrite Nitrogen (NO_2-N)

Both NDFS and ATS showed no significant change before and after treatment (Table 4.4). In NDFS the values before treatment and the one after treatment were 2.29 mg/L and 2.22 mg/L respectively and there was no significant change in the p-value ($p>0.05$). In ATS it was 3.484 mg/L and 4.372 mg/L respectively where p value was statistically insignificant ($p>0.05$). Mean while, in CST, quantity of NO_2-N in the untreated effluent was 6.52 mg/L and in the treated effluent it was 23.22 mg/L. A significant change in the p value ($p<0.05$) was observed where nitrification took place and ammonia was converted to nitrite. However, nitrite values before treatment in NDFS and ATS were below 10 mg/L (General effluent standard) (Table 4.5).

4.5.9 Nitrate Nitrogen (NO_3-N)

Initial values were very negligible in all the three systems 0.1744 mg/L, 0.1784 mg/L and 0.1738 mg/L respectively in NDFS, ATS and CST. Nitrification took place in all the three systems but more amount of NO_3-N was observed in NDFS where it was 5.5100 mg/L in the treated effluent with significant difference in the p value ($p<0.05$). In ATS after treatment it was 1.9040 mg/L with significant change in the p value ($p<0.05$). In CST the after treatment it was 1.0380 mg/Land there was no significant change in the p value ($p>0.05$). However, all the values were below the CPCB general effluent standard less than 20 mg/L (Table 4.5).

4.6 Comparison between the effluent treatment plants

There was no significant change observed in pH in any of the device types and so no comparison. In the case of NO_2 N and NO_3 N, significant change observed only in one device, hence no comparison done between devices. Whereas, statistically significant change could be observed in parameters such as TSS, TS, and O & G, and devices was compared by using ‘T’ test. Statistically no significant change observed in TSS in NDFS and hence compared between ATS and CST. Removal percentage in ATS and CST were 77.9 and 55.68 respectively. Whereas in the case of TS no significant change could observe in CST, so NDFS and ATS were compared by ‘T’ test and found that the removal percentage in NDFS was 72.68 and that in ATS was 85.43%. In the case of O & G significant change was observed in ATS and CST and the removal percentage was 77.92 and 73.6 respectively. These three ETPs differ in their effectiveness in reducing concentrations of parameters of greater concern such as BOD, COD, TSS and NH_3 and the discharged treated wastewater determines the efficiency level of treatment. However, significant change was observed in parameters such as BOD, COD and NH_3 in all the three ETPs and hence compared by ANOVA followed by ‘Tukey’ HSD test. (Table. 4.6).

Comparison between device types which showed significant difference in treatment is done by using’ tukey’ HSD test.

Table 4.6: Percentage removal of BOD, COD and NH_3 in NDFS, ATS and CST

ETP	BOD Means	COD means	NH_3 Means
NDFS	95.56517^{a*}	81.469069^{a}	74.76449 ^{a}
ATS	91.44392 ^{a}	86.72289 ^{a}	56.18357 ^{a}
CST	43.03350 ^{b*}	32.97127 ^{b}	-13.06969 ^{b}

a* - Values within CPCB Effluent Standard Limit
b* - Values not within the CPCB Effluent Standard Limit

Fig.4.3: A modern Effluent Treatment Plant A Conventional Effluent Treatment Plant

4.7 Discussion and Conclusion

Colmenarejo et al. (2006) determined the general efficiency indicator to compare overall performances of the different plants in terms of average TSS, COD, BOD_5 and ammonia removal efficiencies likewise the efficiency of plants is generally measured in terms of removal of organic matter (CPHEEO, 1993).

pH is an important indicator of the contamination of the wastewater, which determines acidity or alkalinity of the effluent. Existence of most biological life is dependent upon narrow range of pH, hence it directly affects performance of a secondary treatment process Metcalf and Eddy (1991 & 2003). During this study it was recorded that pH of the influent as well as effluent was close to neutral. Slightly alkaline pH values of processing waste have also been reported by various researchers Mines and Robertson (2003), Gonzalez J.F (2005).

According to McGhee (1991) solid removal is an important measure for the success of a primary treatment unit. Total solids give an organic load measured as BOD and COD, Molina J. (2003) and have great complications in the wastewater treatment processes. TS were significantly reduced below CPCB Effluent Discharge Standard into the Inland Surface water in NDFS and ATS, whereas in CST there was no considerable reduction. Total dissolved solids are of great concern as it affects the reuse of water for agricultural purpose as it decreases the hydraulic conductivity, if it exceeds 480 mg/L Bouwer (1978). Even though, there was significant difference between the before treatment value and after treatment value of ATS and CST, only the treated effluent from ATS was below the CPCB Effluent Discharge Standards into Inland Surface water. Meanwhile, before treatment value in NDFS was 46.22 mg/L and that was below the CPCB standard (<50 mg/L).

BOD removal is one of the most important criteria to measure the efficiency of biological treatment processes and it is the most reliable parameter for judging the extent of pollution in the water, Mishra and Saksena (1991). Though BOD values in all the three ETPS was significantly reduced in the treated effluent, only in NDFS the BOD value came below the CPCB effluent standard limit (<30 mg/L) (Table 4.5).

COD indirectly measures the pollution strength in wastewater, high COD values may be due to the presence of huge amount of organic matter and this also indicate that the processing effluents contain large amount of biologically resistant substances. Similar results have been observed by Vidal et al. (2000) and Corkum (2003). Treatment efficiency was much higher in both NDFS and ATS where the COD value of treated wastewater was below 250 mg/L, minimum permissible limit of general effluent standard set by CPCB. Before treatment value of oil and grease in all the three ETPs were below the CPCB fish processing industry standard permissible limit (< 10 mg/L) (Table 4.5). Significant difference was observed between treated and untreated effluents in NDFS and ATS, whereas in CST the values in the treated effluent were increased considerably. This might be because of high protein content in the effluent. Ammonia is toxic to aquatic organisms and the oxidation of ammonia to nitrate in receiving water can exert a significant oxygen demand, (Barnes and Bliss, 1983, Rittman and McCarty, 2001). Nitrification took place in all the three ETPs but the value was below CPCB limits (<20 mg/L). The efficiency of the studied plants were calculated by considering the effectiveness of reducing COD, BOD_5, TS, TDS, TSS of the final effluent from each plant.

There is a soaring demand for processed fish products over the world and thereby wastewater load from the seafood processing sector also increasing and the organic content also varies depending on the composition and the operating process.

Based on the discharge water quality analysis from three effluent treatment plants, it can be summed up that both Nano Dispersion and Flocculation System and Aerobic Treatment System are suitable for treatment of seafood processing wastewater in seafood processing industry. The Conventional Septic Tank system is inadequate for treating seafood effluent properly, and discharging this treated effluent to the water body, the whole treatment process becomes obsolete and the water is almost polluted without treatment.

Aerobic Treatment System is an eco friendly and most advanced effluent treatment system which provides high quality treated water tested for BOD and COD. This process is highly fast and it requires 16 hrs to 18 hrs for its batch operation. However, because of high power consumption, (approximately 72 units/day for 100000 liters of effluent) running cost is very high, and its operation requires highly skilled staff to maintain the ETP properly and to keep them is very expensive and the factory owners hesitate to maintain a technically qualified and skilled labor. Compared to ATS, NDFS is the latest and technologically efficient to meet the standards of CPCB. It has less power consumption and a small area is required to install and is easy to operate. The most enticing aspect is that the treated water can be reused in the plant. Initial cost is very high if it set individually, however, cost reduction is possible if set out as a common unit for cluster of plants.

It is of course a welcome approach that almost all the seafood industries of this area has come up with installation of the mandatory treatment plants as insisted by the pollution control board of the state. But these statutory bodies presently have no fool proof system to ensure the operation of these plants and safeguard the environment. The present system of treatment need more cost on operation, time and technically qualified people which in any way is not a pleasant thing for the owners of this industry as they naturally be on the profit side of the industry showing more tendency not to operate well the plants or to discharge the waste water directly to the natural water bodies saving more on the operation cost. It is likely that there will be an installed waste water plant associates with all industries as a testimony of the environmental commitment but seldom has it turned operative for its operation cost.

In recent years several seafood processing plants in the study area have invested considerable amount of resources in building effluent treatment plants (ETP). Unfortunately, many of these facilities have not fulfilled the requirements of the discharge permits. It is more important to conserve energy and resources and therefore more attention is being paid to the selection of processes that conserve energy and resources. Operation and maintenance costs are the prime concern of facility owners. The available technologies are unaffordable due to high energy, capital and maintenance cost. According to the factory owners the present wastewater treatment systems are very expensive and complex. During lean production periods, ETP operation is very expensive. Most of the plants are not able to handle the installed capacity, due to the fluctuating raw material cost and its availability and lack of sufficient labors. The annual production of 46% of the plants was less than 200 tons/month during the last year. The cost for working of effluent treatment plant becomes very expensive, due to the electricity bills, increased maintenance charge and labour charge. Apart from all these, there is the lack of skilled technicians to handle the ETP system. In some places seafood processing takes place in small industrial units which makes it difficult to treat individually and specifically entire waste stream. One of the main reasons for not installing a full capacity waste treatment system is the non availability of the required area. Majority of the plants processes more than one species, making the design of waste treatment facility more complex. Another major problem is that, some of the processors operate only for a part of the year most of the plants process more than one species of fishes, hence to design a waste treatment facility is more complex. While in some other plants which have a high production throughout the year, and the wastewater generated overwhelm the capacity of the effluent treatment plant. Above all, the strict norms and conditions of CPCB and the public protest against pollution, lack of women workers in this sector (mainly because, the women workers joined the Deseeya Thozhilurappu Padhadhi, an undertaking of the Central Government), make the existence of this industry very questionable. So there is a doubtless demand for more advanced and more economically viable wastewater treatment methods.

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Chapter 5

TESTING OF SBSBR TREATMENT SYSTEM TO SUIT THE REQUIREMENTS OF PROCESSING CENTRES

Contents

5.1 Introduction

Unpolluted water is a dire need, not only for human survival, but also for all forms of living organisms and for the ecosystem conservation. However, as human activities increased, so have the wastewater discharge causing the contamination of various habitats. Brisk industrialization has resulted in the surge of pollution consequently havocked environment. For example, around 10,000 new organic compounds synthesized in different industrial activities are discharged each year to wastewater (Metcalf and Eddy 2003), while numerous people suffer the consequences of water scarcity, contamination and inadequate sanitation. The contamination of water bodies and deterioration of natural ecosystems due to pollutants present in the wastewater are major concerns. In developed and developing societies, seafood processing industries are increasingly growing due to stability and market demand as well generated large amount of wastewater (Tay et al., 2006).

Large amounts of industrial fish processing wastes or residues of high nutrient content, which if not properly treated or utilized, is likely to be accumulated in the environment creating pollution and health problems (Hwang and Hansen 1998; Kotzamanis et al., 2001). Fish processing residues include scales, viscera, fish scrap, fat solids, proteins, fish rejects, and liquid stick waste water (UN Report, 1997; Hwang and Hansan 1998). It is due to the high content in soluble organic matter and their different concentrations of suspended solids, depending on the raw materials and the characteristics of industrial process are the major reasons for their pollution effect.

In lieu of high cost of wastewater treatment systems there is an up surging need to develop low cost methods of treating wastewater. To counter the above shortcoming and to preserve the high quality of the environment new concept so called 'Cleaner production' for waste minimization is being introduced, technology designed to prevent waste emission at the source of generation itself (Uwadiae et al., 2011). The most effective way to treat the domestic and industrial wastewater, is to develop low cost wastewater treatment technology, especially in the tropical and subtropical regions (NgMiranda et al., 1989; Puskas et al., 1991; EI-Gohary et al., 1995; Rosen et al., 1998; Larsen et al., 2004)

Some seafood processing wastewater consists of important concentrations of organics and high pollutant loading as Biochemical Oxygen

Demand (BOD_5), Chemical Oxygen Demand nutrients due to a large part contains of residual food waste which is easily biodegradable (Wang et al., 2005; Porntip et al., 2006). Some of the wastewaters from the seafood processing industry, which have high protein content give a high organic nitrogen concentration, that gets converted to ammonium in the anaerobic process which having toxicity above a certain level. (Anderson et. al.,1982 and Veiga, 1989).

Ammonia emission and proteinaceous matter decomposition leads to pH alterations (Gonzalez, 1996). As reported by a few fish processing plants the overall ammonia concentration ranged from 0.7 mg/L^{-1} to 69.7 mg/L^{-1} (Technical Report Series FREMP 1993). In fish condensate the total ammonia content can be up to approximately 2000 mg N L^{-1}. High BOD is generally associated with high ammonia concentrations (Technical Report Series FREMP, 1993). The degree of ammonia toxicity depends primarily on the total ammonia concentration and pH. High ammonia nitrogen concentration causes serious problems such as eutrophication. Unpleasant odour and high temperature are also other issues.

With increasing pollution complexity and high energy consumption, treatment of wastewater becomes complex and uneconomical. Nonetheless, increasing difficulties mean that effluent treatment systems fail to meet sustainability and environmental protection criteria. Thus the industry is faced with increasing problems of waste handling and disposal, plant sanitation, raw material availability and cost, production efficiency and increasing labour and energy cost. If the wastewater treatment and operating costs significantly increase at fish processing plants, some plants could not find it profitable to stay in business. Biological process is one of the most important parts in seafood processing wastewater treatment system which involves removal of non-settleable colloidal solids, stabilization of the organic content, removal of toxic organic compounds and reduce the concentration of inorganic compounds (Rosso et al., 2011).

Many countries find it difficult to reduce the emissions of nitrogen compounds (ammonia, nitrate, NO_x) to the surface waters and the atmosphere. Since mainstream domestic wastewater treatment systems are usually already overloaded with ammonia, a dedicated nitrogen removal from concentrated secondary or industrial wastewaters is often more cost-effective than the disposal of such wastes to domestic wastewater treatment. (Schmidt et. al., 2006).

Wastewater treatment system consists of physical, chemical and biological processes in biological mechanism, in a built environment; living organisms like bacteria enhance their functions. Biological wastewater treatment has traditionally been based on empirical approaches, where the microbes are generally treated in uncertainties. Most bacterial species present in wastewater have not yet been cultivated in any laboratory (Caballero, 2011).

Since the seafood effluent is rich in nutrients, biological treatment methods are quite suitable for treatment. A number of biological treatment systems have been developed to treat high strength wastewater generated by food industries. The most common method in biological treatment is activated sludge process. With stringent regulations of effluent discharge, conventional activated sludge alone is unable to remove the pollutants of wastewater in desirable limits. On the other hand, chemically treated wastewater generated a compound and complex effluents in addition to achieve high sludge retention (Aziz et al., 2011).

Aerobic methods are considered to be the best with lower economic costs (Gavrilescu et al., 1999 and Metcalf and Eddy, 2003). These processes allow the removal of large amounts of soluble biodegradable COD in the concentration range up to 4000mg/L (Grady et al., 1999). Use of microorganisms to degrade organic compounds into inorganic products is the basis of biological treatment methods. It can be classified as attached growth process and suspended growth. In the attached growth system it uses a medium to retain and grow microorganisms. For example, Trickling Filters (TF), Rotating Biological Contactors (RBC), Fluidized Bed Reactor (FBR), Fixed Film Bioreactor (FFBR) and Flexible Fibre Bio film reactors. Where as in the suspended growth systems are completely mixed flocculent processes where, microorganisms are in the suspension have more intimate contact with the substrate. Aerated Lagoons (AL), Sequencing Batch Reactor (SBR) and Activated Sludge Process (ASP) are certain examples. Residual ammonia is considered to be an obstinate problem in effluent treatment plants dealing with protein-rich wastes. The tenable mechanism for ammonia removal from effluent treatment plants is biological nitrification, in which microorganisms have an important role involving ammonia (NH_3) oxidation to nitrate (NO_3) via nitrite (NO_2) (Keluskar et al., 2013).

Disposal of effluents from seafood processing industries pause a major threat, because of their high protein and ammonia contents. Industry is in dire need of efficient system of wastewater treatment, which covers the mechanism and process used to treat protein and ammonia rich waters prior to release into the environment (Devivaraprasad et al., 2014). Biological treatment based on nitrification-denitrification is the best option for nitrogen removal in such waters. NH_4^+ being discharged in aquatic ecosystems has been proven toxic to life when present in its un-ionized form (NH_3) and can cause eutrophication (Arthur et al., 1987; Hall, 1986). The relative concentrations of ionized and un-ionized forms of ammonia depend on pH and temperature (Emerson et al., 1975).

5.2 Biological nitrogen removal (BNR)

Nitrogen compounds such as NH_4^+, NO_2^- and NO_3^- can be removed from wastewater biologically and conventionally performed through the combined biochemical system of nitrification (aerobic) and denitrification (anoxic).

5.2.1 Nitrification

Nitrification is the biological oxidation of reduced forms of nitrogen, usually ammonium to nitrate. The group of bacteria of the family, Nitrobacteriaceae (Buchanan; 1917) collectively known as nitrifiers, which undertake the nitrification reaction, include two discrete microbial partners tied faithfully to a life of biochemmical harmony, namely ammonia oxidizers (nitritifiers) and nitrite oxidizers (nitratifiers). Both partners firmly depend on each other. To remove nitrogenous components, the process of nitrification and de nitrification are normally applied (Breisha et al., 2010).

Nitrification or ammonia oxidation is the oxidation of ammonia to nitrite and then nitrite to nitrate by autotrophic bacteria (Ebling et al., 2006). In the first stage, oxidation occurs and ammonia is converted to nitrite by a group of autotrophs and in the second stage nitrites further oxidized to nitrate by another group of autotrophs (Vymazal, 2007). Nitrifying bacteria are the autotrophic microorganisms that obtain energy from the oxidation of reduced nitrogen. Some species of heterotrophic bacteria, fungi, actinomycetes and archaea (Focht and Chang, 1975, Park et al., 2006) have been reported to perform nitrification. However the oxidation mechanism that takes place in wastewater treatment is considered to be mainly carried out by autotrophic ammonia - oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) respectively (Koops and Moeller, 1992., Bock and Koops, 1992). Monophyletic chemolythotropic ammonia oxidizing bacteria (AOB) are believed to be completely responsible for aerobic ammonia oxidation (AO) being the first and rate limiting step of nitrification. (Kowalchuk and Stephen, 2001). The biological process of nitrification involves conversion of toxic ammonia (NH_3) to non-toxic nitrate (NO_3) through the action of autotrophic nitrifying bacteria and can be expressed as follows

$$2NH_4 - +3O_2 \longrightarrow 2NO_2 - +4H+ +2H_2O + \text{energy}$$

$$2NO_2+O_2 \longrightarrow 2NO_3- + \text{energy}$$

Nitrosomonas (AOB) and Nitrobacter(NOB) have been the genera responsible for nitrification in wastewater treatment. However, recent studies show a much higher diversity, with other genera involved (Purkhold et al., 2000; Limpiyakorn et al., 2005, Siripong and Rittmann, 2007). It is the temperature; pH and DO are the main parameters affecting the process of nitrification, apart from sludge retention time, (SRT) which is an operational parameter. As reported by Siripong and Rittmann in (2007) different temperatures seem to change the AOB consortia compositions and the diversity of AOB has also proven to be affected by temperature shifts (Park et al., 2009).

AOB are ecologically important, being the only group of organisms that oxidize ammonia to nitrite in significant amounts, and they appear to be present in all environments in which nitrogen is mineralized (Aakra et al., 1999). Because these microorganisms display low growth rate and high sensitivity to environmental disturbances and inhibitor, the physiological activity and abundance of AOB in wastewater processing is important in the design and operation of waste treatment systems.

From an operational point of view, the nitrification has its optimum in the pH range 7.5 – 8 (Metcalf and Eddy, 2003). Nitrifying bacteria have been highly susceptible to the presence of toxic compounds in wastewater and can either decrease or stop nitrification rate after a wash-out period. However, when they are present in complex systems like activated sludge basin, they are relatively resistant to toxicity (Metcalf and Eddy 2003).

5.2.1.1 Factors Affecting Nitrification

5.2.1.2 Oxygen Tension

Every milligram of nitrogen passed through their full nitrification pathway from ammonia to nitrite requires approximately 4.5 mg of dissolved oxygen (Alleman and Preston 1991), to scavenge electrons drawn from their nitrogenous substrates. If there is a drop in dissolved oxygen below a few mg per litre, nitrifier metabolism will remarkably slowdown.

5.2.1.3 pH and Alkalinity

Nitrifiers prefer an alkaline pH range between 7-8 to proceed nitrification smoothly, and alkalinity levels adequate to stop pH from dropping below the preferred alkaline range is required.

5.2.1.4 Temperature

Nitrifying bacteria prefer moderate to warm temperatures, ranging from 28-38^{o}C. The growth constants of nitrifying bacteria were affected greatly by temperature (Sharma and Ahlert 1997).

5.2.1.5 Salinity

They have a sizable range of tolerable osmotic pressures, ranging from fresh to saline, depending on the genus. Many can switch rapidly from one salt level to another with little input on their activity. A peculiar characteristic is concentration of salts (Na^{+}, Cl^{-}, SO_4^{2-}, K^{+}, Ca_2^{+}, Mg_2^{+}) which may lead to two types of effluents (Mendez et.al; 1988).

1) High salinity wastewaters when the salts come from the products and /or the utilization of seawater in the process.
2) Low salinity wastewaters, when fresh water is used as a process water and no salinity is produced by the product.

Among these ions sodium, chloride and sulphate in high concentrations can cause inhibition or toxicity effects during the biological treatment (Mendez, et.al., 1989, Soto et al., 1991).

5.2.1.6 Light

Nitrifying bacteria are sensitive to visible and long wavelength u.v light. Photo inhibition has been demonstrated to be due to photo oxidation of cytochrome C (Bock, 1965).

5.3 Denitrification

The reduction of NO_3- to N_2 gas is known as de-nitrification and occurs in nature under anoxic conditions. As NO_3 act as electron acceptor, it is reduced to NO_2^- , Nitrous oxide (N_2O), Nitric Oxide (NO) and N_2. The enzyme involved in the first step of de-nitrification is nitrate reductase. This is a molybdenum containing membrane –integrated enzyme which is expressed only when NO_3 is present and under the absence of DO.

Literature survey reveals that there are a number of novel approaches to biological treatment of the ammonia or total nitrogen, have been developed in recent years. Such as SHARON (Single Reactor High Activity Removal Over Nitrite), ANAMMOX (Anaerobic Ammonia Oxidation), CANON (Completely Autotrophic Nitrogen Removal over Nitrite), OLAND (Oxygen Limited Aerobic Nitrification-Denitrification) and so on, on a laboratory scale as well as pilot plant study.

In this research a laboratory scale study was conducted to treat wastewater generated from seafood processing plants by using nitrifying bioreactor. A survey of literature revealed that the use of SBSBR in treating seafood effluent is a novel and unique method and extensive research has to be done to use it for commercial purposes.

- The aim of the present work was to determine the potential use of SBSBR to treat seafood effluents. The main objective of this research work was to establish connections between wastewater treatment process parameters and use of Stringed Bed Suspended Bioreactor

5.4 Materials and Methods

5.4.1 Stringed Bed Suspended Bioreactor (SBSBR)

(PCT Patent application No. 828/DEL/2000/India) a technology commercialized through M/s Oriental Aquamarine Biotech India (P) Ltd., Coimbatore, India.

5.4.1.1 Configuration of stringed bed suspended bioreactor (Kumar et. al., 2009)

The reactor has four components: (1) an outer shell of 10 cm^3 with conical bottom; (2) an inner cartridge comprising a solid frame work and beads on strings with filter plates both at its top and bottom; (3) an airlift pump at the center of the filter plates; and (4) a black lid with perforations on top. Based on study by Achuthan (2000), polystyrene and low density polyethylene beads of 5 mm diameter and a surface area of 0.785 cm^2 with spikes on the surface had been selected as the substrata for immobilizing ammonia and nitrite oxidizing consortia, respectively. On full assembly the cartridge with beads was inserted into the outer shell and the black lid was placed on top. The beads stringed in the reactor cartridge provided an overall surface area of >684 cm^2 to support the nitrifying biofilm in addition to the inner surface of the shell and cartridge framework.

5.4.2 Detrodigest

A bioremediation technique in which a novel microbial preparation composed of *Bacillus sp*; has been designed for Indian aquaculture sector and is technically named as 'DETRODIGEST'. The organism used for the preparation of Detrodigest is *Bacillus cereus* sensu lato MCCB 101 (Genbank Acc. No. EF 062509) sequestered from aquaculture fields of

Kerala and manipulated to rigorous screening procedures. It has validated to be a relevant preparation for prolonged and safe detritus management in aquaculture system. In a comprehensive study on the salinity preference of the organism in Detrodigest revealed its euryhaline nature by growing and adequately producing hydrolytic enzymes at all salinities tested ranging from fresh water to seawater (0 - 45 ppt) (Haseeb, 2012). *Bacillus sp.* in Detrodigest is highly accomplished with the potential to produce a variety of enzymes such as protease, lipase, chitinase etc. Its capability to bring down ammonia is a distinguished property of this organism. Rapid degradation of detritus as soon as formed by Detrodigest makes more dissolved oxygen.

5.4.3 Wastewater Samples

Four types of wastewater samples were used in the experiment. Wastewater sample from:

1) cuttlefish processing unit
2) fish filleting facility
2) shrimp processing unit
3) shrimp pre processing (peeling shed) unit.

5.5 Experimental Set up

Cuttlefish processing effluent from a seafood processing facility was collected at close to the end of the pipe prior to entering the main treatment system usually during peak processing time, typically late morning, in two cleaned cans of volumetric size 25L, and transported to the laboratory. The samples were thoroughly mixed, measured 20 L and poured into two rectangular fiber tanks of size 25 L which were named as 'Test' and 'Control'. A portion of the sample was used for analyzes of different parameters such as pH, Ammonia, nitrite, nitrate, dissolved oxygen (DO), biological oxygen demand (BOD_5), chemical oxygen demand (COD). The pH was measured by pH meter. While NH_3, NO_2, NO_3 were analyzed by UV-Vis Spectrophotometer, TSS by filter Glass Fiber Filter Disc, DO and BOD_5 by Winkler's method, and COD by Potassium Dichromate Digestion (APHA).

The 'Test' and 'Control' tanks with 20 L of effluent were continuously aerated. pH maintained between 7 - 8 by the addition of Sodium bicarbonate (Na_2CO_3) in the test tank. The addition of sodium carbonate provides suitable conditions for the nitrifying bacteria by avoiding potential restrictions in the use of inorganic carbon, one of their substrates. Effluent in the test tank was supplemented with 'Deterodigest^{TM} '. Subsequently Stringed bed suspended bioreactor (SBSBR) was introduced (Kumar et al., 2009).

Samples from both the tanks were analyzed daily for various parameters, such as ammonia, nitrite, nitrate, pH, Chemical oxygen demand (COD) and biological oxygen demand (BOD) were analyzed on a daily basis.

The same methods were repeated with wastewater samples from fish filleting, shrimp processing and shrimp pre processing units.

The data collected for the study were compiled and analyzed statistically by two way ANOVA followed by Tukey's HSD test and the level of significance was set at $P<0.05$.

Table 5.1: Selected physical, chemical and biological characteristics commonly analyzed in wastewater treatment and their significance in terms of process design and performance Characteristic Significance

Temperature	To design the most suitable biological process
Ammonia (NH_4^+) Organic Nitrogen (Org N) Nitrites (NO_2^-) and Nitrates (NO_3^-) Total Nitrogen Total Phosphorous (TP)	A measure of the nutrients and degree of decomposition of a wastewater
pH	A measure of the acidity or basicity of a wastewater
Biological Oxygen Demand (BOD) Chemical Oxygen Demand (COD) Total Organic Carbon	Different parameters to measure the organic content of a wastewater

Adapted from Metcalf and Eddy, 2003

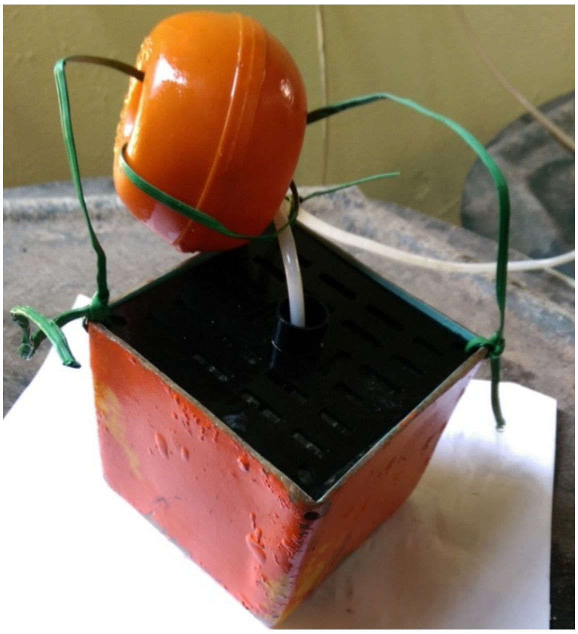

Fig. 5.1: Stringed bed suspended bioreactor

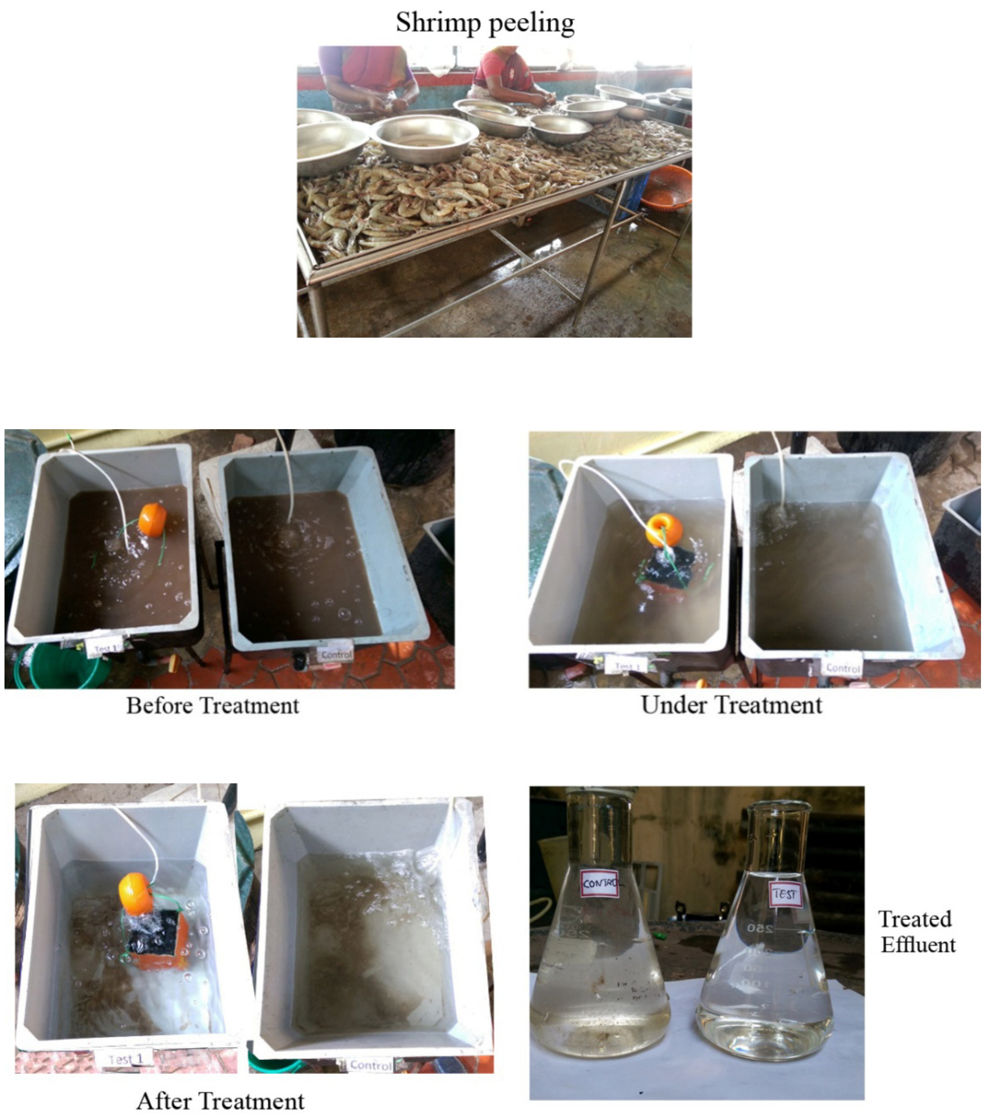

Fig. 5.2: Experimental set up

Fig. 5.3: Experimental set up 2

5.6 Results and Discussion

5.6.1 Colour and Odour

Effluent colour from both shrimp processing and pre-processing facility appeared pale red, but the peeling shed effluent is more turbid, this may be because of less volume of water is used while peeling. Fish filleting effluent was red in color because of the presence of blood and shredded muscles, where as the cuttlefish effluent seemed to be dark black ashy colour and all these have foul and obnoxious odours.

After treatment, the color of each effluent turned to clear, transparent and agreeable, especially in the test than the control.

5.6.2 pH

It is one of the most important pollution indicators in the water quality analysis, which expresses the intensity of acidity or alkalinity. According to Wiesmann et al. (2007), Rogalla et al. (1990) and Ahmed et al. (2007), the microorganisms responsible for nitrification develop better in slightly alkaline condition in the range from 7.2 to 8.0. The nitrifying bacteria may be more sensitive to temperature and pH Hargrove et al. (1996). pH of the wastewater should be maintained Akpore et al. (2008). pH was controlled in all the test tanks by adding sodium bicarbonate for the optimum growth of bacteria. According to Ferreira (2000), Jianlong and Ning (2004), the nitrification rate can drop significantly for pH value below the neutral zone. Control of pH within the growth optimum of microorganisms may reduce ammonia toxicity (Bhattacharya and Parkin, 1989). Study of Sandberg and Ahring (1992) claimed that when pH was increased slowly to 8.0 or more

COD removal drop about 15- 17%. Hence pH was maintained 7- 8 by adding sodium bicarbonate.

5.6.3 Ammonia NH_3 –N

There was significantly lower concentration of ammonia in the test tank compared to control ($P<0.05$) in all the four types effluent after treatment, and an increase in concentration was observed in the initial days of treatment. The increase of both ammonia nitrogen and TKN concentrations could be attributed to the anaerobic bioconversion of proteins which contained into amino acids and then to ammonia Demirer and Chen (2005). Such results were agreed with Aziz et al. (2011) and Roy et al. (2010). In shrimp processing effluent the initial concentration of ammonia in both the tanks were 61.41 mg/L. The initial increase in ammonia concentration was observed in all the four experiments and later stabilized and reduced gradually this agrees with Manahan (2005), which was indicating the occurrence of nitrification in the reactor (Fig. 5.4 to 5.7). Significant change was observed on the fifth (5^{th}) day of treatment in the shrimp processing effluent (Table.5.6), and in cuttlefish processing effluent (Table.5.9), whereas it took seven (7) days each for pre-processing (Table 5.7) and fish filleting (Table.5.8) effluent. The high removal of ammonia at the beginning of the experiment indicated that the nitrifying bacteria growth in the reactor was occurred Ujang et al. (2002). 100% removal of ammonia was observed on the 12^{th} day of treatment in the fish filleting test sample. On that particular day mean value of ammonia in the control was 78.4 mg/L (stdv ±0.56). On the 10^{th} day of treatment ammonia was zero in the shrimp pre-processing test sample, whereas, the mean value of ammonia in the control was 74.6 mg/L (stdv. ± 1.21). In the shrimp processing effluent test sample value of ammonia became zero on the 17th day, while in the control sample it was 72.7 mg/L (stdv.± 0.80). On the 20th day of treatment ammonia became zero in the cuttlefish processing test sample, whereas, the mean value of ammonia in the control sample was 72.7 mg/L (stdv.± 0.85). The highest removal efficiency was observed with ammonia in the test sample. The organic matter was utilized by microbes through oxidation process and biosynthesis of new microorganisms. (Fontenote et al., 2007) There was a progressive reduction in the organic matter and increase in NO_2-N and NO_3-N in the test tank with the removal of ammonia agreed with. Initial ammonia concentration in all test samples was above 55 mg L^{-1}. After the treatment, ammonia in all test samples were reduced to 0, whereas, there was no considerable reduction in the control samples (Fig. 5.4 to 5.7).

5.6.4 Nitrite (NO_2-N)

Nitrification in the test tank was found established instantly and progressed rapidly in the test samples than in the controls. There was a significant reduction in the conversion of ammonia to nitrite in the test samples, whereas, a slow and gradual increase in the nitrite formation in the control samples (Fig. 5.8 to 5.11). Nitrite is an intermediate compound of nitrification (Hargreaves, 1998). Mean while in the test samples with reactor, significantly low quantity of NO_2-N found to have built up; instead, there was a steady increase of NO_3-N. (Fig. 5.12 to 5.15). This showed the establishment of the two- step nitrification in the test tanks with reactor. A significant change between test and control observed on the fifth day of the treatment in shrimp processing ($P<0.001$) (Table 5.6) and in shrimp pre processing ($P<0.001$) (Table 5.7) effluent. In cuttlefish processing effluent in the test tank significant change was observed on the fourth day of the treatment (Table 5.7) and in fish filleting it was on the seventh day of the treatment. There was a correlation between the duration and treatments in all the four experimental setups (Table 5.2 to 5.5) and significant change was observed between test and control. Nitrite formation was much more in the control tank than in the test (Fig. 5.8 to 5.11). The rate of nitrite remained below 10 mg/L in all the test samples throughout the experiment, nitrite build up was very small, not gradually increasing as expected, considering that nitrate is the end product of nitrification (Hargreaves, 2006).

5.6.5 Nitrate Nitrogen (NO_3-N)

A considerable increase in the conversion of ammonia to nitrate was observed in all the experimental set ups. By analyzing ANOVA Table 5.2 to 5.5, there was significant interaction between the treatments $P<0.05$ (Test and Control) and between duration and treatments.

Formation of Nitrate was established on the third day in the shrimp processing (Fig 5.12) (Table 5.6) and cuttlefish processing (Fig. 5.15) (Table 5.9) test sample with reactor. $P< 0.001$. In the peeling effluent (Fig. 5.13) and filleting (Fig. 5.14) test tank nitrate formation was established by the sixth and fifth day respectively and a noticeable difference was observed between test and control ($P<0.001$). Analysis of Fig. 5.12 to 5.15 a significant increase in NO_3-N could see in all the test samples with SBSBR reactor, which showed the efficiency of the reactor in nitrification. By comparing figures of ammonification and nitrification it was very clear that when there is a reduction in ammonia concentration subsequent increase in nitrate nitrogen (NO_3-N) occurred. In all biological systems nitrate gets reduced to nitrogen gas by denitrifying bacteria under anoxic conditions as in anaerobic sediments. (Hargreaves, 1998).

5.6.6 Dissolved Oxygen (DO)

Nitrifiers are strict aerobes, nitrification is inhibited at very low DO levels. If the D O >2mg/L, there is little impact on nitrifier growth. DO concentrations have a direct effect on growth rate of nitrifying bacteria Pramanik et al., (2012). By analyzing Table 5.2, (Fig. 5.16) there was a significant change in the DO concentration as the treatment proceeds. There was significant difference between the DO in test sample with reactor, compared to control sample in the shrimp processing effluent and the mean value of dissolved oxygen in the test sample was 7.2 mg/L (± 0.11) and in the control was 6.7 mg/L (± 0.1157) ($P<0.01$). In the peeling effluent there was no statistically significant difference observed between test and control (Table 5.3) (Fig. 5.17) the mean value of DO in the test sample with reactor was 6.25 mg/L (± 0.2) and in the control was 6.15 mg/L (± 0.2) ($P>0.05$). In the fish filleting test sample, significant change was observed on the seventh day of the treatment (Table 5.8), a gradual reduction in DO concentration (Fig. 5.18) this may be because of intense nitrification process in the test tank at that period (Fig. 5.15) and the mean value observed was 6.06 mg/L (± 0.115) and in the control was 5.93 mg/L (± 0.115) ($P>0.05$) (Table 5.4) (Table 5.8). This agrees with Balmellae et al. (1992) found that insufficient DO level can result in a buildup of nitrite in the effluent. Nitrification rate is increased with increasing DO and is found to be almost linearly dependent upon the oxygen concentration up to more than 10 mg/L Kermani et al. (2009), Quasim (1998). Approximately 4.33 mg of O_2 are consumed per mg of NH_4^+ oxidized to nitrate (Benefield and Randall, 1985). However, in cuttlefish processing test sample there was significant difference observed with that of the control (Table 5.5) and on the 17th day there was a significant reduction in the DO concentration (Table 5.9) (Fig. 5.20). The mean value was 6.4 mg/L (± 0.23) and in the control it was 5.93 mg/L (± 0.1154) ($P<0.05$).

5.6.7 Biological Oxygen Demand (BOD_5)

BOD is the most reliable parameter for judging the extent of pollution in the water (Mishra and Saksena, 1991). The high BOD value could be probably due to the presence of more organic material in wastewater (Molina, 2003 and Islam et al., 2004). Ammonia nitrogen affected on BOD_5 in wastewater during transformation NH_3 into NO_3- due to its biologic oxidation process by nitrifying bacteria, which might have a significant dissolved oxygen requirement (Wang et al., 2005). Significant reduction in BOD observed in all the four types of effluents treated. By analyzing Table 5.2 to 5.5 there was statistically significant reduction in the BOD in the test compared to control $p<0.001$ in all the experiments. Duration has significant impact on BOD reduction $p<0.05$. Significant change in BOD was established on the 7th day in shrimp processing effluent $p<0.05$ where the initial BOD was 1708 mg/L and on the seventh day it was 910 mg/L (Table 5.6), and in fish filleting effluent $p<0.05$, where the initial value was 2933 mg/L and on the 7th day it was 955 mg/L (Table 5.8), initial BOD value in shrimp pre-processing effluent was 3800 mg/L and on the eighth day it was 1066 mg/L ($p<0.05$) (Table 5.7) and in cuttlefish processing effluent initial value was

1802 mg/L and on the sixth day it reduced to 905 mg/L a significant reduction from the initial value was observed ($p<0.05$) (Table 5.9). Finally, mean value of shrimp processing effluents treated by bioreactor was 12.5 mg/L (± 6.25) lower than the industry specific standard set by CPCB (<30 mg/L) and in the control sample it was 70 mg/L (± 7.2) ($P<0.05$) (Fig.5.20). Mean value of BOD in the shrimp preprocessing effluent was 27.6 mg/L (± 4.6) and that in the control was 151.2 mg/L (± 74). In fish filleting test tank mean value was 23 mg/L (± 5.7) and in the control 76.8 mg/L (± 8.8). Mean value of cuttlefish effluent 14.4 mg/L (± 1.7) and that in the control was 416 mg/L (± 14.4). The percentage removal of BOD in the treated samples were 99%, whereas in the control, the removal percentage in shrimp processing 95.8%, in peeling effluent it was 96%, in fish filleting effluent 97% and in cuttlefish 76%.

5.6.8 Chemical Oxygen Demand COD

It is **c**ommonly used to indirectly measure the amount of organic pollutants in wastewater. Statistically more significant reduction in COD observed in all the test samples than in control samples. By analyzing Table 5.2 to 5.5 observed a significant reduction in COD $p<0.05$ in the test tank with reactor than in control tank. Significant reduction took place in shrimp processing effluent where the initial COD was 2933 mg/L and on the 7th day of treatment it was reduced to 1200 mg/L ($p<0.05$) (Table 5.6), and in fish filleting the initial value was 4666 mg/L and on the 7th day of treatment it was reduced to 1937 mg/L ($p<0.05$) (Table 5.8), considerable reduction was observed on the 8th day in peeling effluent where the initial value was 4800 mg/L and was reduced to 2000 mg/L ($p<0.05$) (Table 5.7) and in cuttlefish processing effluent the initial value was 2896 mg/L and on the 6th day it was reduced to 1200 mg/L ($p<0.05$) (Table 5.9). Finally, the mean value of shrimp processing effluent in the test sample 133 mg/L (± 45) and in the control 373 mg/L (± 46), in the peeling effluent the mean value was 160 mg/L (0) and in the control 320 mg/L (0). Mean value in the fish filleting effluent was 186.6 mg/L (± 46) and in the control 746.6 mg/L (± 46) and the mean value for cuttlefish 106 mg/L (± 45) and in the control tank 603 mg/L (± 5.7). After treatment COD in the test tank with reactor came below CPCB standard < 250 mg/L. The removal percentage of COD in all the test samples were above 95%, whereas in control it was 87%, 93%,84%,and 79% in shrimp processing, shrimp peeling, fish filleting and cuttlefish processing effluents respectively.

5.7 Conclusion

Nitrifying activity of suspended bacteria has been reported to be very slow because of slow growth rate and inhibition of nitrification by free ammonia and nitrite under alkaline conditions of seawater, in biological ammonia removal system (Bower and Turner, 1981; Furukawa et al, 1993). It takes 2-3 months to establish nitrification in marine systems without the addition of nitrifiers as start – up cultures. (Manthe and Malone, 1987) and in fresh water it requires 2-3 weeks (Masser et al., 1999). Under such condition immobilization techniques have been found useful to overcome the delay in the initiation of nitrification (Sung-Koo et al., 2000).

SBSBR may be used as treatment process for nitrogen and COD removal to meet discharge requirements in the seafood industries. It can be used as a compact system for small scale processors and pre processors if it is developed fully. It is anticipated that it could reduce the capital and operational cost, this is justified not only by its treatment performance but also by economic considerations as the application of the most advanced treatment technologies are limited to high capital or operational costs. Apart from that, sludge disposal is a major problem faced by processors; it can be solved to a certain extent by the addition of Detrodigest which convert the organic matter for their cell metabolism as carbon and energy source. The removal of large organic particles reduces water turbidity (Haung, 2003).

In general, it can be used to treat high strength organic wastewaters like seafood processing effluents. Total nitrogen concentration can be vary from low to high depend on the type of raw material processed and process type, which require an appropriate treatment process for the removal. Attractive feature of the technology is that the system can be put under operation as per the requirements with intermittent 'on' and 'off' as per the availability of effluent to be treated. During the 'off' period the reactor can be kept idle with just under aeration by feeding with ammonium sulphate and pH adjustment. Application of advanced treatment methods or processes will vary depending on the location availability of land, social and economic concerns. Thus this innovative bioreactor need to be evaluated and make use of its potential and applied properly for the protection of our environment and resources.

Table 5.2: ANOVA showing the effect of treatments (Test and Control) and duration in shrimp processing effluent.

Effluent Type	Parameters	Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
SHRIMP PROCESSING EFFLUENT	NH_3	Duration	17	47114	2771	6322	<2e-16	***
		Treatments	1	110506	110506	252092	<2e-16	***
		Duration: treatments	17	11453	674	1537	<2e-16	***
		Residuals	72	32	0			
	NO_2	Duration	17	3798	223	830.5	<2e-16	***
		Treatments	1	4432	4432	16475.8	<2e-16	***
		Duration: treatments	17	1759	103	384.8	<2e-16	***
		Residuals	72	19	0			
	NO_3	Duration	17	9033	531	2585	<2e-16	***
		treatments	1	10747	10747	52289	<2e-16	***
		Duration: treatments	17	9555	562	2735	<2e-16	***
		Residuals	72	15	0			
	BOD_5	Duration	3	10019868	3339956	1744.503	< 2e-16	***
		Treatments	1	39447	39447	20.604	0.00034	***
		Duration: treatments	3	20132	6711	3.505	0.03988	*
		Residuals	16	30633	1915			
	COD	Duration	3	26200212	8733404	8187.6	< 2e-16	***
		treatments	1	398610	398610	373.7	1.62E-12	***
		Duration: treatments	3	157111	52370	49.1	2.71E-08	***
		Residuals	16	17067	1067			
	DO	Duration	3	1.698	0.5661	37.741	1.74E-07	***
		treatments	1	0.135	0.135	9	0.00848	**
		Duration: treatments	3	0.325	0.1083	7.222	0.00279	**
		Residuals	16	0.240	0.0150			
	pH	Duration	17	28.76	1.692	1.265	0.2393	
		treatments	1	14.55	14.552	10.88	0.0015	**
		Duration: treatments	17	3.59	0.211	0.158	0.9999	
		Residuals	74	98.8	1.38			

Table 5.3: ANOVA showing the effect of treatments and duration in shrimp pre processing effluent

Type	Parameters	Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
SHRIMP PRE PROCESSING	NH_3	Duration	19	141456	7445	237.01	<2e-16	***
		Treatments	1	57562	57562	1832.49	<2e-16	***
		Duration: treatments	19	15854	834	26.56	<2e-16	***
		Residuals	80	2513	31			
	NO_2	Duration	19	8190	431	627.1	<2e-16	***
		Treatments	1	8202	8202	11932.3	<2e-16	***
		Duration: treatments	19	10711	564	820.1	<2e-16	***
		Residuals	80	55	1			
	NO_3	Duration	19	10263	540	2039	<2e-16	***
		Treatments	1	4862	4862	18352	<2e-16	***
		Duration: treatments	19	13660	719	2714	<2e-16	***
		Residuals	80	21	0			
	BOD_5	Duration	3	47869663	15956554	17956.72	< 2e-16	***
		Treatments	1	29372	29372	33.054	2.99E-05	***
		Duration: treatments	3	12223	4074	4.585	0.0168	*
		Residuals	16	14218	889			
	COD	Duration	3	67315200	22438400	1.85E+32	<2e-16	***
		Treatments	1	2457600	2457600	2.02E+31	<2e-16	***
		Duration: treatments	3	2380800	793600	6.53E+30	<2e-16	***
		Ressiduals	16	0	0			
	DO	Duration	3	0.5733	0.19111	10.424	0.000481	***
		Treatments	1	0.06	0.06	3.273	0.089268	.
		Duration: treatments	3	0.4333	0.14444	7.879	0.001884	**
		Residuals	16	0.2933	0.01833			
	pH	Duration	19	121.31	6.385	121.73	< 2e-16	***
		Treatments	1	1.29	1.29	24.59	3.91E-06	***
		Duration: treatments	19	15.78	0.831	15.84	< 2e-16	***
		Residuals	80	4.20	0.052			

Table 5.4: ANOVA showing the effect of treatments and duration in fish filleting effluent

Type	Parameters	Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
FISH FILLETING EFFLUENT	NH_3	Duration	19	149615	7874	151.81	<2e-16	***
		Treatments	1	117207	117207	2259.57	<2e-16	***
		Duration: treatments	19	26878	1415	27.27	<2e-16	***
		Residuals	80	4150	0.0117			
	NO_2	Duration	19	2822	148.5	409.3	<2e-16	***
		Treatments	1	1270	1269.7	3499.5	<2e-16	***
		Duration: treatments	19	2871	151.1	416.5	<2e-16	***
		Residuals	80	29	0.4			
	NO_3	Duration	19	9403	495	872.9	<2e-16	***
		Treatments	1	9738	9738	17177.1	<2e-16	***
		Duration: treatments	19	12185	641	1131.2	<2e-16	***
		Residuals	80	41	1			
	BOD_5	Duration	3	29011731	9670577	2106.68	< 2e-16	***
		Treatments	1	1044502	1044502	227.54	7.02E-11	***
		Duration: treatments	3	947960	315987	68.84	2.31E-09	***
		Residuals	16	73447	4590			
	COD	Duration	3	55593333	18531111	902.489	< 2e-16	***
		Treatments	1	1344267	1344267	65.468	4.79E-07	***
		Duration: treatments	3	512800	170933	8.325	0.00146	**
		Residuals	16	328533	20533			
	DO	Duration	3	0.9783	0.3261	27.952	1.34E-06	***
		Treatments	1	0.015	0.015	1.286	0.274	
		Duration: treatments	3	0.085	0.0283	2.429	0.103	
		Residuals	16	0.867	0.0117			
	pH	Duration	19	30.299	1.5947	136.207	<2e-16	***
		Treatments	1	0.071	0.0708	6.044	0.0161	*
		Duration: treatments	19	7.559	0.3978	33.981	<2e-16	***
		Residuals	80	0.937	0.0117			

Table 5.5: ANOVA showing the effect of treatments and duration in cuttlefish processing effluent.

Type	Parameters	Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
CUTTLEFISH PROCESSING	NH_3	Duration	17	44358	2609	4830	<2e-16	***
		Treatments	1	123886	123886	229303	<2e-16	***
		Duration:treatments	17	11416	672	1243	<2e-16	***
		Residuals	72	39	1			
	NO_2	Duration	17	3655	215	811.5	<2e-16	***
		Treatments	1	4251	4251	16043.1	<2e-16	***
		Duration:treatments	17	1754	103	389.4	<2e-16	***
		Residuals	72	19	0			
	NO_3	Duration	17	8814	518	3458	<2e-16	***
		Treatments	1	9384	9384	62574	<2e-16	***
		Duration:treatments	17	9365	551	3673	<2e-16	***
		Residuals	72	11	0			
	BOD_5	Duration	3	10782763	3594254	14096	<2e-16	***
		Treatments	1	1298085	1298085	5091	<2e-16	***
		Duration:treatments	3	890752	296917	1164	<2e-16	***
		Residuals	16	4080	255			
	COD	Duration	3	24667951	8222650	14253.1	<2e-16	***
		Treatments	1	2376489	2376489	4119.4	<2e-16	***
		Duration:treat	3	1291322	430441	746.1	<2e-16	***
		Residuals	16	9230	577			
	DO	Duration	3	4.38	1.46	92.21	2.60E-10	***
		Treatments	1	2.042	2.0417	128.95	4.56E-09	***
		Duration:treatments	3	0.978	0.3261	20.6	9.67E-06	***
		Residuals	116	0.253	0.0158			
	pH	Duration	17	12.411	0.73	6.21	1.53E-08	***
		Treatments	1	18.763	18.763	159.591	< 2e-16	***
		Duration:treatments	17	7.405	0.436	3.705	5.04E-05	***
		Residualls	71	8.347	0.118			

The Dunnet's multiple comparisons showing the day on which significant difference observed in SBSBR with that of control.

Table 5.6: The Dunnet's multiple comparisons showing the effect of treatment duration on SBSBR treated shrimp processing effluent.

Parameter	Days compared	Estimate	Std. Error	t value	Pr (>t)
NH_3[a*]	5 – 1	-9.270	0.544	-17.040	<1e-04 ***
NO_2[b]	5 – 1	2.121e+00	3.463e-01	6.125	<0.001 ***
NO_3[b]	3 – 1	6.013e+01	4.601e-01	130.69	<e-04 ***
BOD_5[a]	7 – 1	-797.67	36.96	-21.58	<1e.7 ***
COD[a]	7 – 1	-1733.33	26.67	-65.00	<2e-16 ***
DO[a]	18 – 1	0.53333	0.09428	5.657	<0.001 ***

*a – Significant decrease, b – significant increase

Table 5.7: The Dunnet's multiple comparisons showing the effect of treatment duration on SBSBR treated shrimp preprocessing effluent.

Parameter	Days compared	Estimate	Std. Error	t value	Pr (>t)
NH_3[a]	7 - 1	-9.4133	0.5414	-17.39	<2e-16 ***
NO_2[b]	5 - 1	2.263e+00	2.859e-01	7.917	<0.001 ***
NO_3[b]	6 - 1	1.223e+01	5.408e-01	22.610	<0.001 ***
BOD_5[a]	8 - 1	-2734.0	15.9	-171.9	<2e-16 ***
COD[a]	8 - 1	-02.800e+03	5.796e-13	-4.831e+15	<2e-16 ***

Table 5.8: The Dunnet's multiple comparisons showing the effect of treatment duration on SBSBR treated shrimp processing effluent on Fish filleting Effluent

Parameter	Days compared	Estimate	Std. Error	t value	Pr (>t)
NH_3[a]	7 - 1	-22.5333	0.5669	-39.751	<0.001 ***
NO_2[b]	7 - 1	1.23863	0.29100	4.256	<0.001 ***
NO3[b]	5 - 1	2.754e+00	7.700e-01	3.577	0.00642 **
BOD_5[a]	7 - 1	-2666.67	55.07	-35.92	<2e-16 ***
COD[a]	7 - 1	-2000.0	134.7	-14.85	<1e-07 ***
DO[b]	7 - 1	0.46667	0.8165	5.715	<0.001 ***

Table 5.9: The Dunnet's multiple comparisons showing the effect of treatment duration on SBSBR treated shrimp processing Cuttlefish processing Effluent

Parameters	Days compared	Estimate	Std Error	T Value	Pr(>t)
NH_3[a]	5- 1	-1.1233	0.3091	-3.634	0.00543***
NO_2[b]	4- 1	2.900e-01	7.3050e-02	3.970	0.00426***
NO_3[b]	3- 1	2.574e+00	6.050e-02	42.548	0<0.001***
BOD_5[a]	6- 1	-897.00	14.33	-62.6	<2e-16***
COD[a]	6- 1	1696.67	19.81	-85.66	<2e-16***
DO[a]	17- 1	0.53333	0.09428	5.657	<0.001***

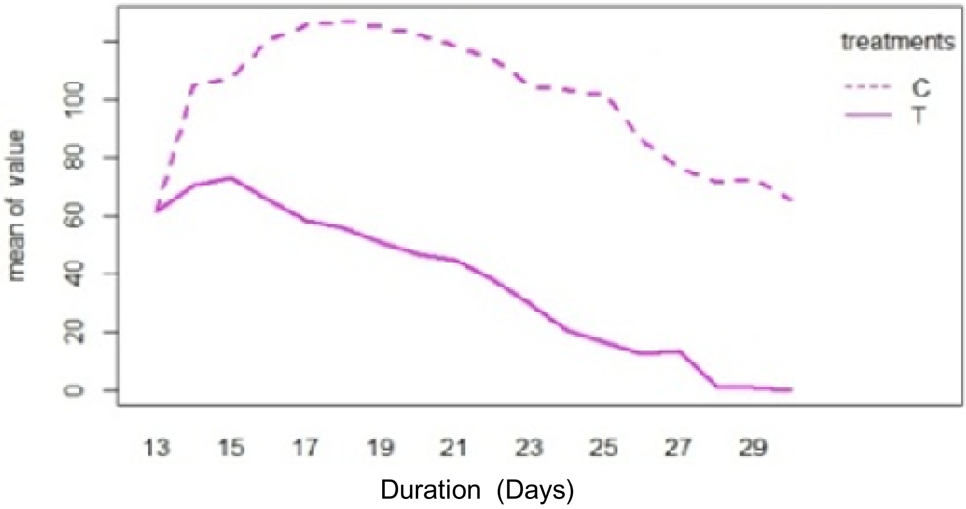

Fig. 5.4: Effect of treatment in ammonia reduction in test and control of Shrimp processing effluent

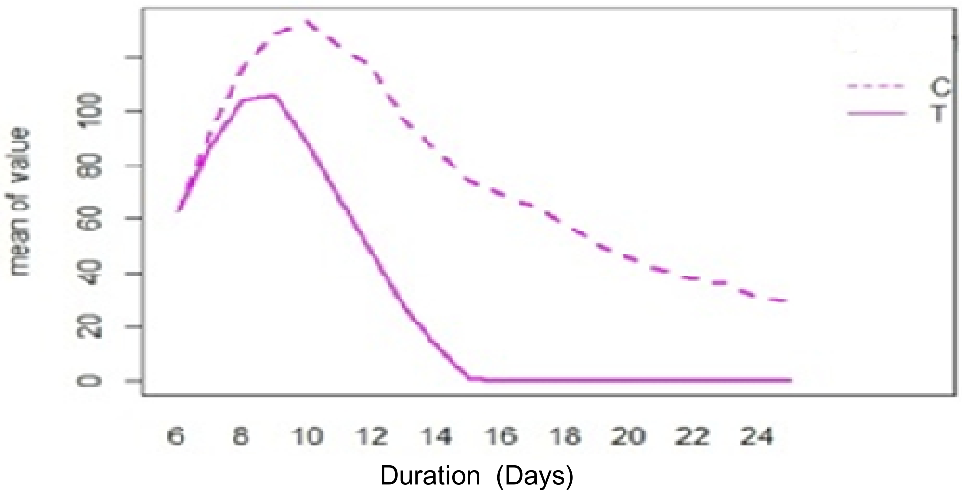

Fig. 5.5: Effect of treatment in ammonia reduction in test and control of Shrimp peeling effluent

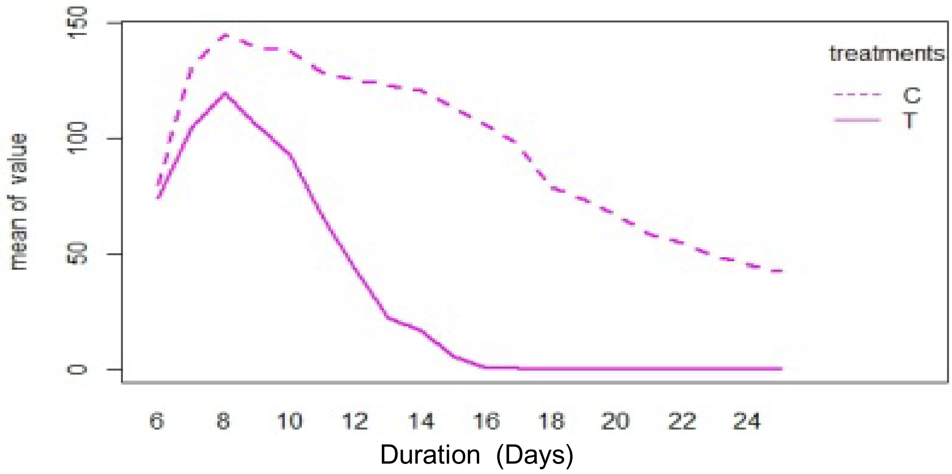

Fig. 5.6: Effect of treatment in ammonia reduction in test and control of fish filleting effluent

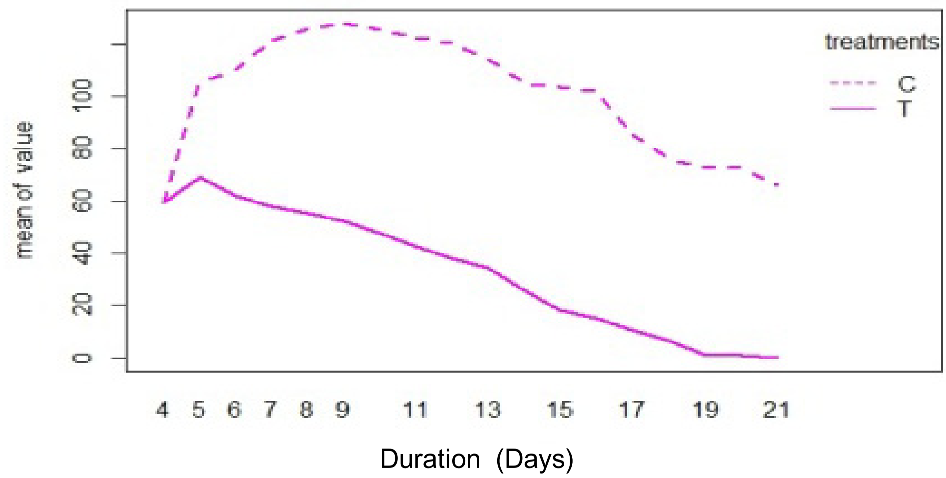

Fig. 5.7: Effect of treatment in ammonia reduction in test and control in cuttlefish processing effluent

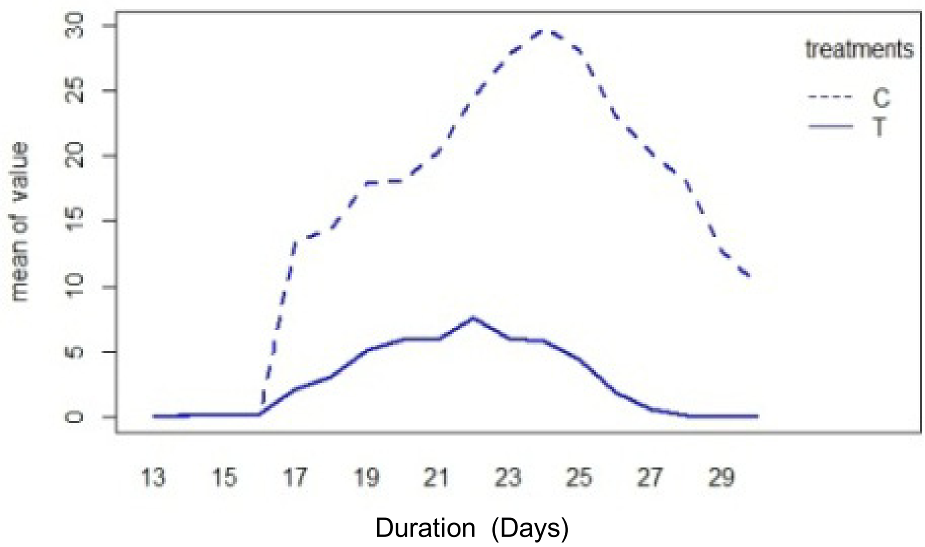

Fig. 5.8: Effect of treatment on nitrite formation in Test and Control of Shrimp processing effluent

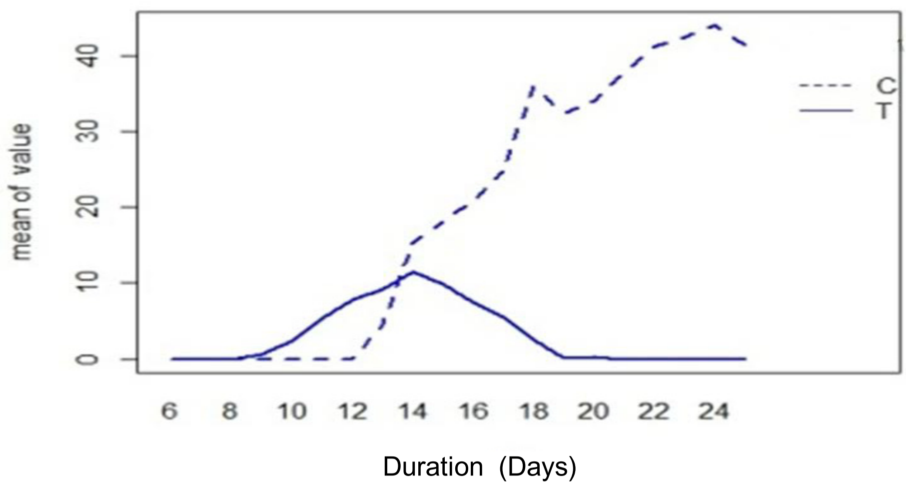

Fig. 5.9: Effect of treatment on Nitrite formation in Test and Control of Shrimp Peeling Effluent

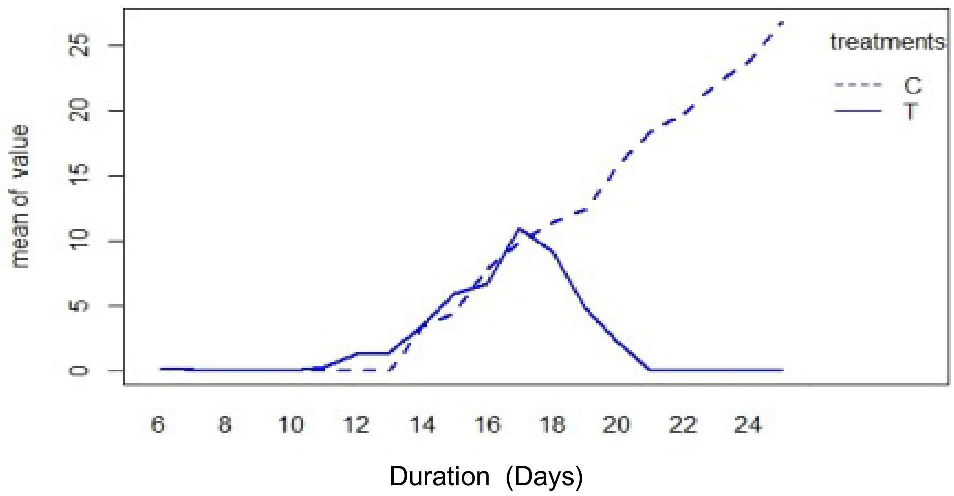

Fig. 5.10: Effect of treatment on Nitrite formation in Test and Control of Fish filleting effluent

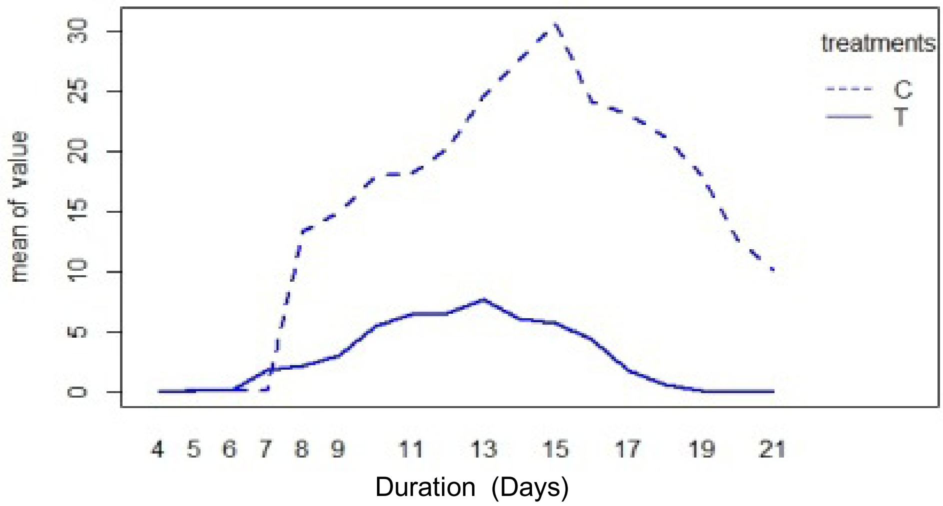

Fig. 5.11: Effect of treatment on Nitrite formation in Test and Control of Cuttlefish processing effluent

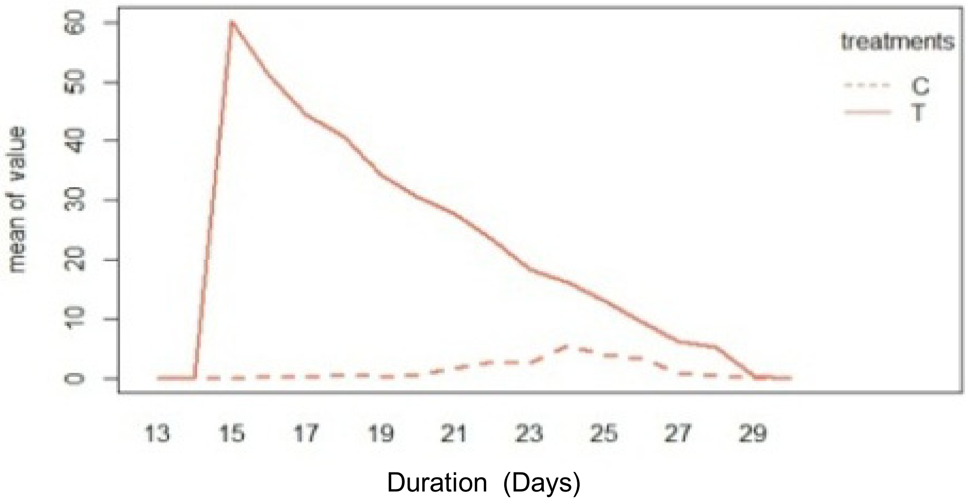

Fig. 5.12: Effect of treatment on Nitrate formation in Test and Control of Shrimp processing effluent

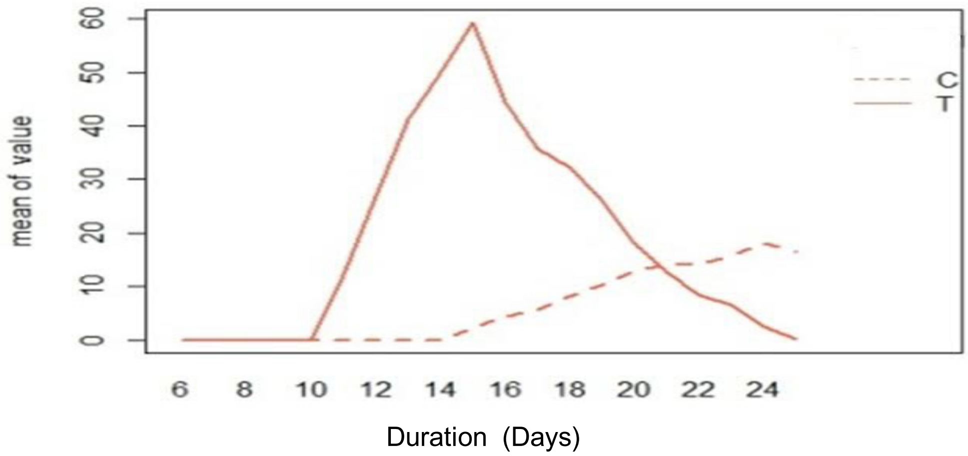

Fig. 5.13: Effect of treatment on Nitrate formation in Test and control of Shrimp peeling effluent

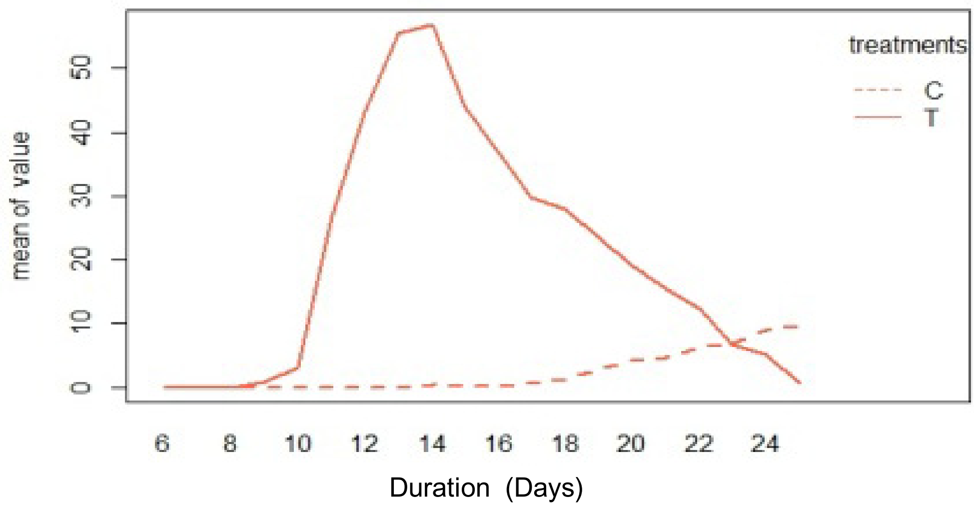

Fig. 5.14: Effect of treatment on Nitrate formation in Test and Control of Fish filleting effluent

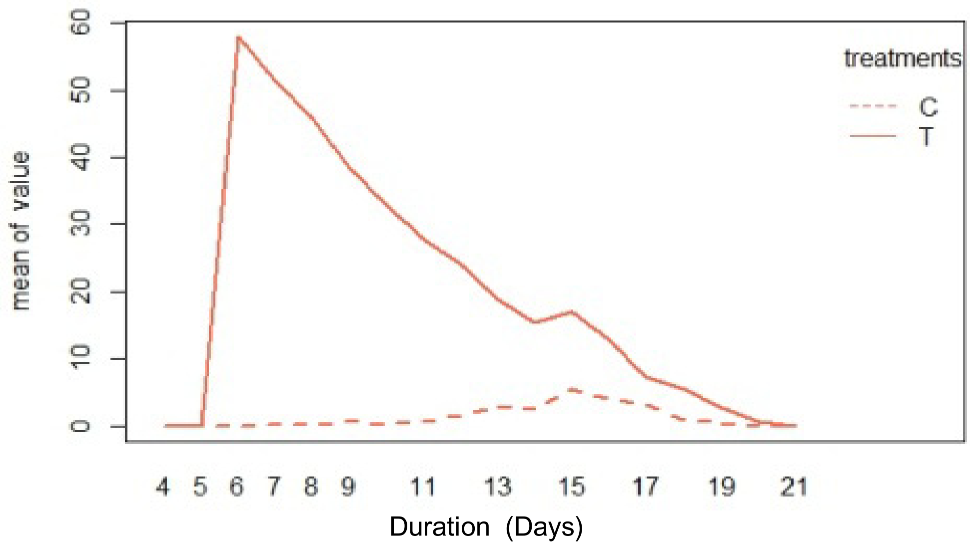

Fig. 5.15: Effect of treatment on Nitrate formation in Test and Control of Cuttlefish processing effluent

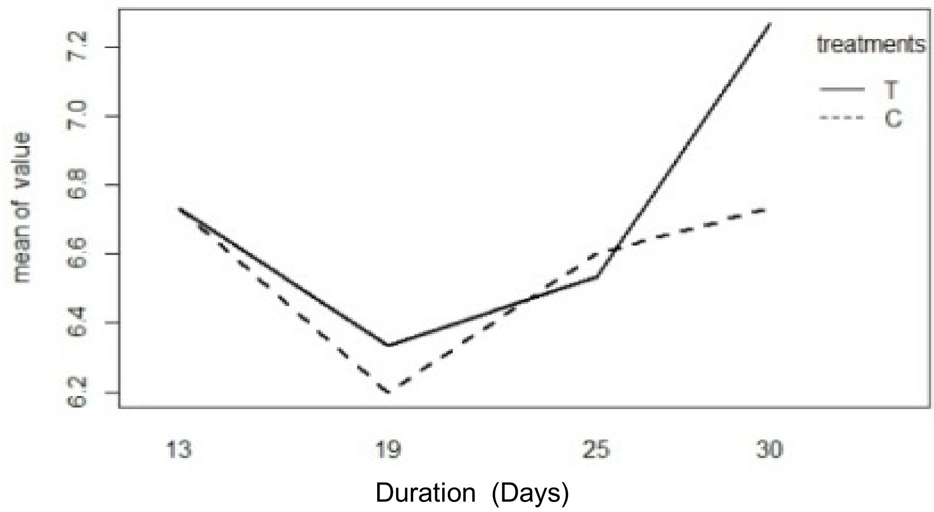

Fig. 5.16: Effect of treatment on D O concentration in Test and Control of Shrimp processing effluent

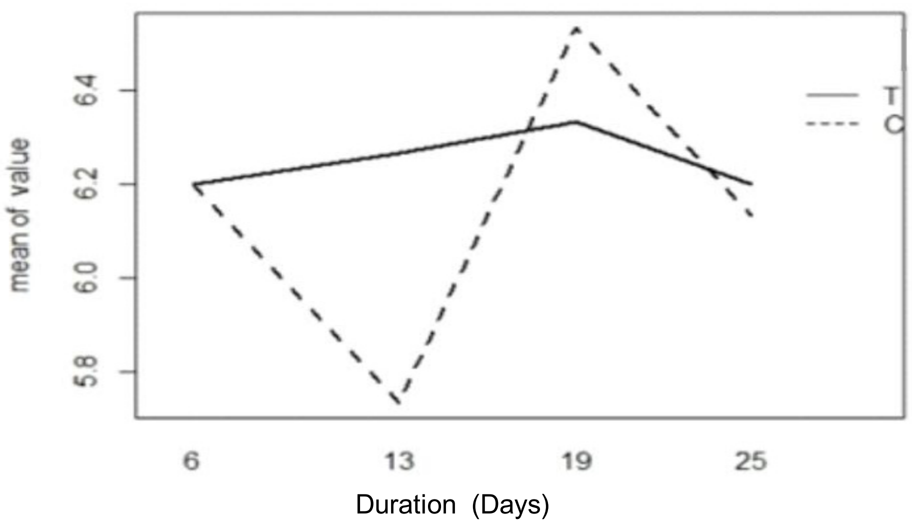

Fig. 5.17: Effect of treatment on D O concentration in Test and Control in Shrimp peeling effluent

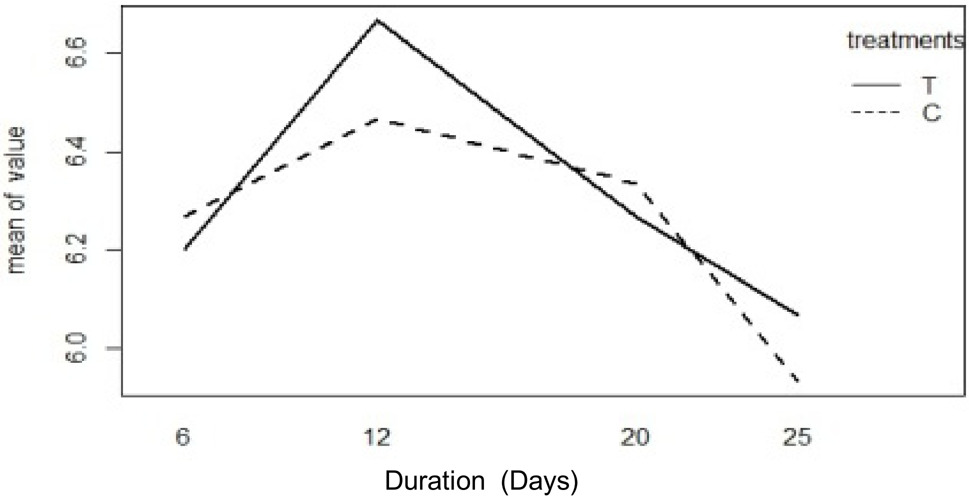

Fig. 5.18: Effect of treatment on D O Concentration in Test and Control of Fish filleting effluent

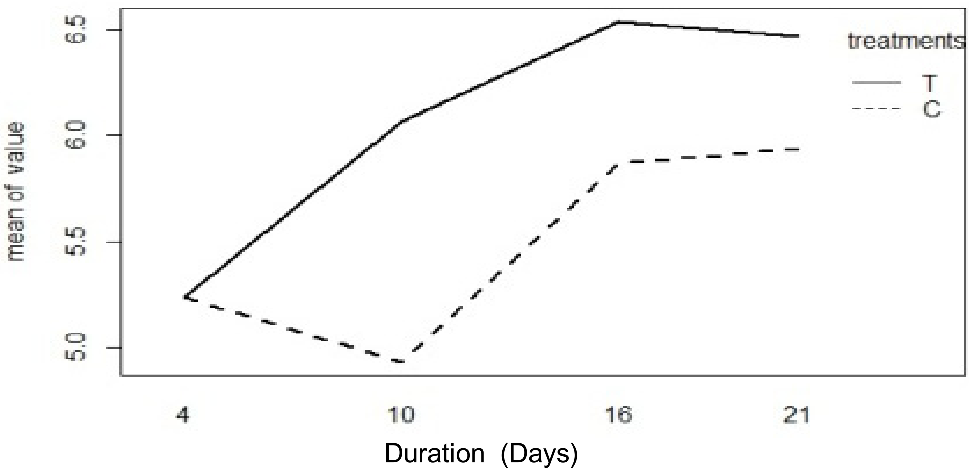

Fig. 5.19: Effect of treatment on D O Concentration in Test and Control of Cuttlefish processing effluent

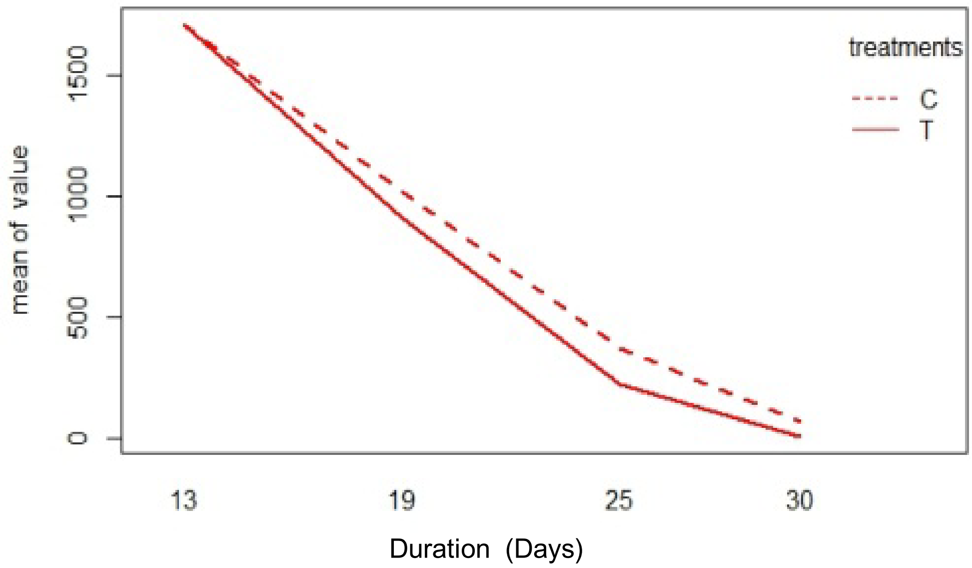

Fig. 5.20: Effect of treatment on BOD removal in Test and Control of Shrimp processing effluent

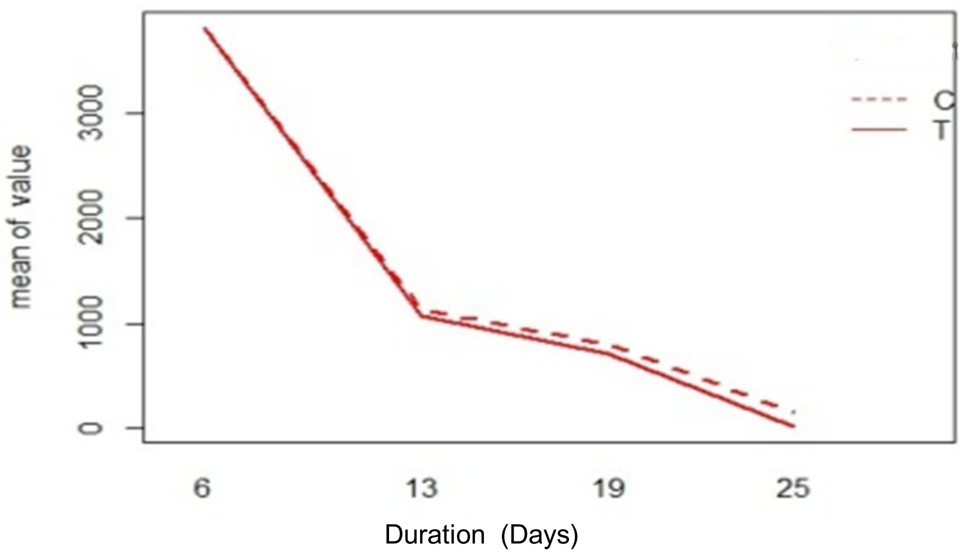

Fig. 5.21: Effect of treatment on BOD removal in Test and Control of Shrimp peeling effluent

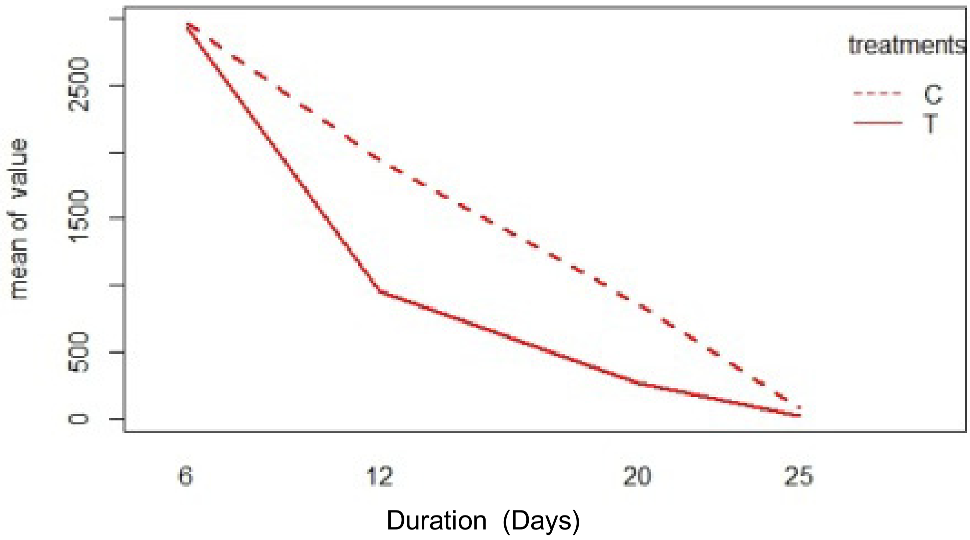

Fig. 5.22: Effect of treatment on BOD removal in Test and Control of Fish filleting effluent

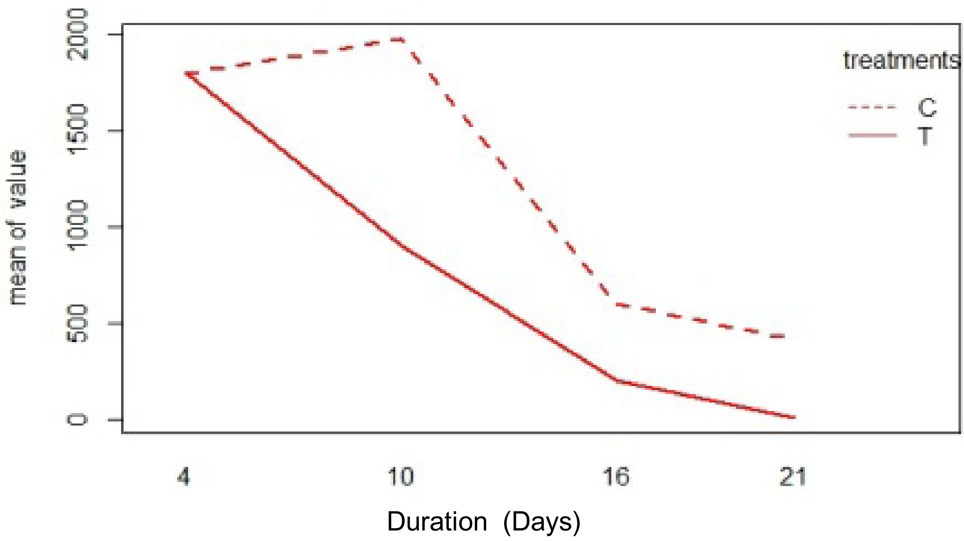

Fig. 5.23: Effect of treatment on BOD removal in Test and Control of Cuttlefish processing effluent

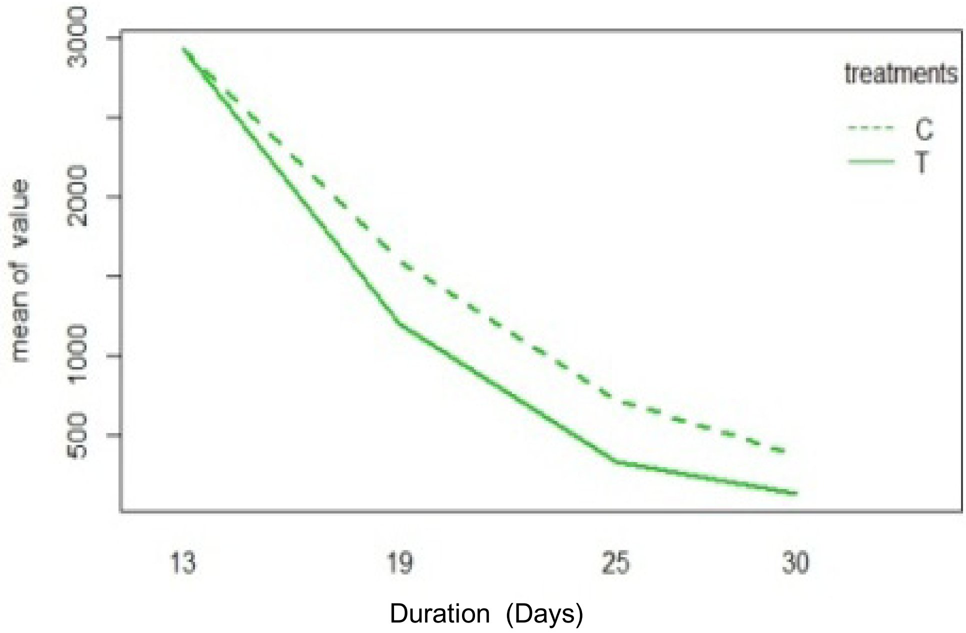

Fig. 5.24: Effect of COD in Test and Control of Shrimp processing effluent

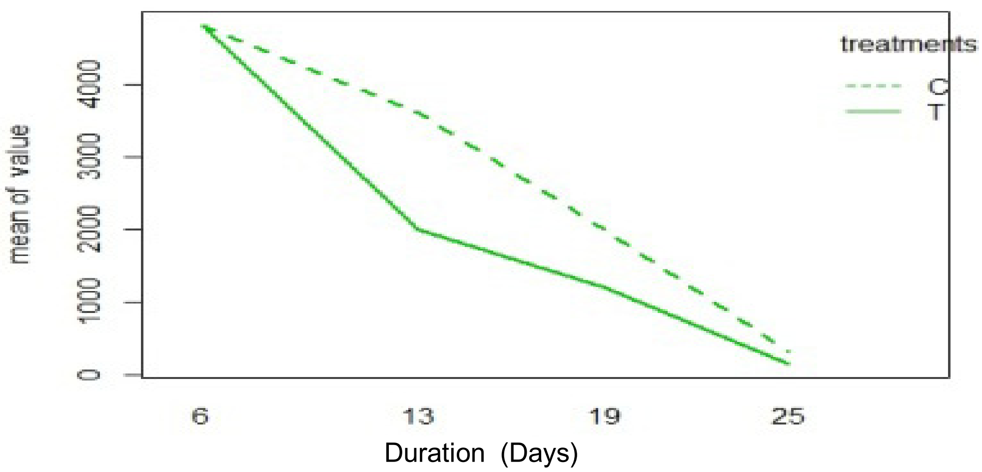

Fig. 5.25: Effect of COD in Test and Control of Shrimp peeling effluent

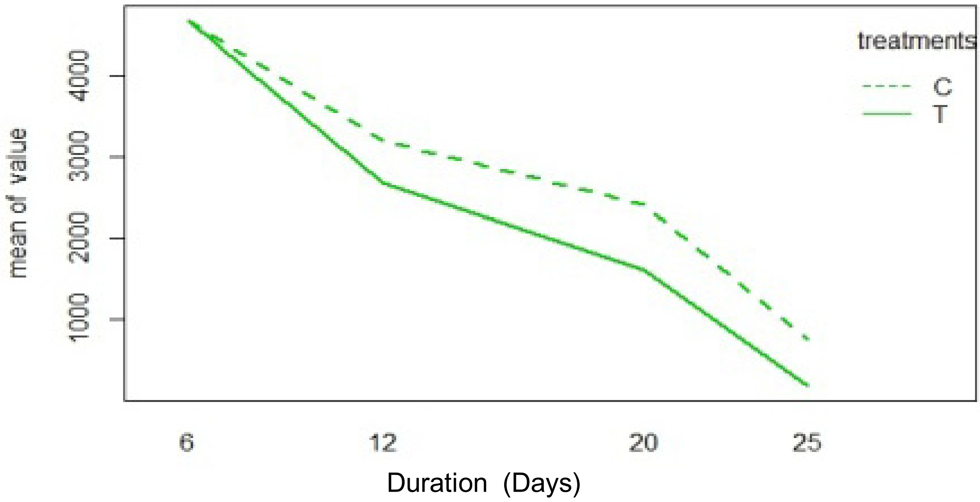

Fig.5.26: Effect of COD in Test and Control of Fish filleting effluent

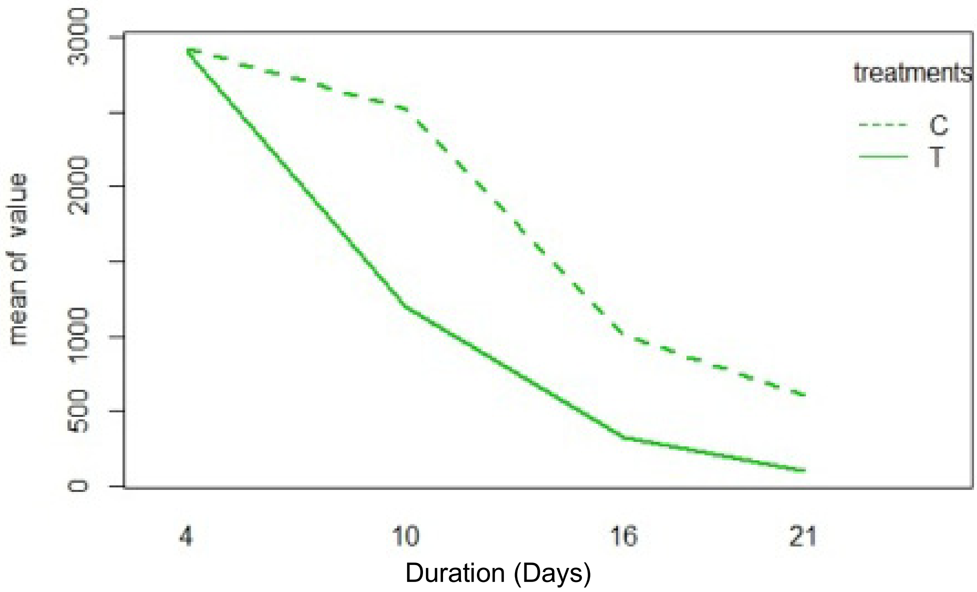

Fig. 5.27: Effect of COD in Test and Control of Cuttlefish processing effluent.

Chapter 6

SUMMARY, CONCLUSION & STRATEGY FOR WASTEWATER MANAGEMENT

The study affirms that seafood processing industry is the backbone of Indian economy at the same time this industry is the inefficient users of water and cause havoc to the ecosystem. Whenever food is handled or processed there is an inherent generation of wastes in the form of solid and liquid. Several technologies were introduced for the management of waste generated, among that organic effluents were of prime importance. As time passed by there was an increase in the percentage of waste production due to inefficient management in harvesting, pre processing and processing. As a result the water bodies inundated with organic pollutants from seafood industries, one of the major issues which requires immediate attention. This study discusses the export potentials of seafood industry, waste generations, waste management practices, and introduces a laboratory scale reactor to treat the seafood effluents.

Chapter 1

A general introduction pointing the present scenario of global, Indian as well as Kerala seafood industry, a brief introduction of the issues of seafood processing effluent was given in the light of growing concern on the pollution potential of the seafood processing sector in India. The chapter discusses the problem, significance of the study area, as well as the area of special interest. Scope and significance of the study discussed as it pioneers an issue based analysis of effluent waste management in Aroor region, Kerala, one of the top seafood processing zones in the country. The objectives of the study were mentioned as to understand the present processing technologies, waste generation and treatment facilities; characterization of the liquid waste generated in the fish pre processing and processing centres; assessment of the efficacy of the existing treatment system and testing of SBSBR treatment system for processing centres. Methodology of the study which is used to achieve different objectives was also discussed in this chapter.

Chapter 2

Results of the survey conducted in the study area collected the primary data enables to understand the present processing technologies, source and type of raw materials used, nature of processing and end products, type of ETPs in the study area, volume of fresh water used and effluent generated etc. were collected through the questionnaires specifically structured for pre processing and processing centres. Maximum production took place in the months of August to December, this period cephalopods surpassed shrimp and finfish. Whereas, in the months of January to May shrimp dominate in production. Shrimps, cephalopods and finfish formed the major seafood varieties processed in this area. All the 20 surveyed plants equipped with one or the other type of ETP. NDFS, ATS and CST are the ETPs found in the study area. Out of 10 peeling shed surveyed only two have ETP and the rest have only settling tanks as the treatment system.

Chapter 3

This chapter introduced by review of literature on the status of physicochemical analysis of seafood effluents and literature regarding by product recovery from seafood processing effluents and a brief explanation on each physicochemical parameters. Effluent samples were collected from four different sites where diverse raw materials were processed. Samples were analysed on a monthly basis for six months Physicochemical parameters such as pH, TS, TSS, BOD, COD, NH_3. Heavy metals such as Hg, Pb, Cd, microbiological characters such as total coliform and faecal coliform were analysed by APHA protocols. Output data was analyzed statistically and found significant difference in the physicochemical parameters of shrimp peeling effluent, shrimp processing effluent, cuttlefish processing effluent and fish filleting effluent. There was significant difference between months. Heavy metals such as lead and cadmium detected. Presence of Coliform indicates the presence of pathogenic microbes in effluent and if not adequately treated it may cause deleterious impacts on the ecosystem.

Chapter 4

This chapter intended to assess the efficiency of the existing ETPs that are prevalent in the study area. Chapter introduced with the literature survey on effluent treatment methods that are followed in seafood industry, followed by a comparative study of three ETPs; NDFS, ATS and CST. Collected samples from the inlet and outlet of ETPs were analysed for important parameters and statistically analysed the obtained data. Results showed that, both NDFS and ATS are suitable for treating seafood effluent where as CST showed no significant change in the after the treatment values.

Chapter 5

The objective of the study was to develop a laboratory scale cost effective treatment system. To achieve this, a patented reactor (SBSBR) was tested with different seafood effluents along with a bioaugmentor. Two tanks each with 20 litres of cuttlefish processing effluent named as ‘Test’ and ‘Control’ and were aerated continuously. Test tank was supplemented with ‘Detrodigest’ a microbial preparation capable to rapidly degrade off organic matter and make available more dissolved oxygen. Subsequently, Stringed Bed Suspended Bioreactor was introduced. pH was maintained between 7-8 as it was the optimum pH for the nitrifying bacteria. Samples were analysed on a daily basis for parameters that have specific concerns by PCBs of centre and state. Statistical analysis of data showed that there was significant difference in the parameters between the test and control samples. Ammonia in the test tank was found reduced to zero, whereas in control, it was in at higher concentration (66 mg/L). Same was observed with NO_2-N, when nitrite formation was completed in the test tank it was 12 mg/L in the control tank, when nitrate formation (NO_3-N) was completed in the test tank it was just began in the control tank. When BOD was reduced below CPCB limit (<30 mg/L) 14.4 mg/L in the test tank with reactor, it was 416 mg/L in the control tank, same was observed with COD when it was 106 mg/L in the test tank with reactor, in the control tank it was 603 mg/L . This showed the effectiveness of the reactor in treating the seafood effluent. Same was repeated with shrimp pre processing and processing effluent and fish filleting effluent, observed significant difference between the test and the control sample and found that the reactor was effective to treat diverse seafood effluents and could be adopted as a simple and economically viable, with low operational complexities and suitable for small scale processing industries and pre processing centres.

From the study it is elicited that seafood effluent has to be subjected to energy efficient and low cost technologies before discharging to the aquatic ecosystem. This low cost technology would support small scale processors and pre processors who are presently facing many challenges such as productivity increase and production cost reduction and are under pressure to improve the environmental footprint and hygiene especially in the context of export potential. A low cost and energy efficient treatment system would save the residents those who are presently suffering from foul smell and breathing problem, ground water pollution, fish mortality that affects their livelihood, crop destruction by wastewater infiltration, stagnated and clogged canals that are the breeding place for vectors.

Future perspectives:

- This lab scale reactor should be extended to a full scale plant so that it can be applied in the industry
- More engineering aspects have to be combined with this reactor so that it can functions on its full potential.
- Further studies need to be conducted to know its limitations and potentials so that it can be applied in other fields of effluent treatment.

Strategy for wastewater management in seafood processing plants

Findings in the study were analysed to develop a strategy for wastewater pollution abatement by considering the following steps:

- Preventive strategies to reduce pollution load
- Use of economically viable, that effective at reducing pollution to CPCB standard.

Preventive strategies to reduce pollution load

Seafood industry is an incompetent user of water and use large amount of water for washing and cleaning and also for glazing and freezing. Apart from that it is a good medium of transport by act as lubricant in various steps of processing and handling. In addition to the high organic content in the effluent it also contains a variety of detergents and disinfectants, which are applied during cleaning activities. It contains an array of chemicals include acid, alkali and neutral detergents and disinfectants consists of chlorine compounds, hydrogen peroxide and formaldehyde. If we reduce this contaminant load may increase the ETP efficiency, and hence solutions from the processing floor has to prevent prior to the entry into the ETP effluent stream and thereby could reduce the TSS and BOD.

Considering this, the following strategies are suggested:

- Instead of flushing with water convey the offal and food grade materials mechanically
- Design the process line so that different effluent stream can be kept separate to permit appropriate treatment options.
- Use a dry vacuum system for the dry removal of offal
- Before wet cleaning by using hose conduct a dry cleaning
- Floor drains and collection channels should fit with grids and screens
- Outlet of wastewater channels should fit with screens and fat traps to reduce the amount of solids wastes.
- Collect viscera and other organic materials separately for by product utilization
- Choose detergents that have no adverse impacts on wastewater treatment processes and sludge quality
- Optimize their use to a correct dosage

- Avoid disinfectants with active chlorine
- Isolate high organic strength wastewater from the diluted effluents.
- Install drip pans or trays to collect drips and spills
- Avoid submersion of fillets in water, as soluble protein may leak out and enter the effluent stream.

To reduce water consumption the following steps can be suggested

- Use high pressure rather than high volume
- Use compressed air instead of water
- Install water meters on high use equipments
- Report and fix leaks promptly
- Construction of floors and walls easy to clean
- Dry clean-up is a major step to reduce water consumption.

Treatment of waste water in an economically feasible manner

Install Common Effluent Treatment Plants for a cluster of industries (CETP). It is practically possible in Aroor area where a number of plants are in close proximity. This can reduce the installation cost, maintenance cost and reduction in energy and resource use.

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* As available from the source material.

Appendix

SCHOOL OF ENVIRONMENTAL STUDIES

Cochin University of Science and Technology

Research Project Evolve a Technically & Economically feasible effluent treatment system for seafood Processing Industries

QUESTIONNAIRE

1 NAME OF THE FIRM	
2 NAME OF THE LOCALITY	
3 CATEGORY	
4 NAME OF THE PRODUCTS	FISH,PRAWN,CUTTLE FISH, SQUID, OCTOPUS,CRAB
5 SPECIFY BYE PRODUCTS IF ANY	
6 RAW MATERIALS USED	FISH, CUTTLE FISH, SQUID, PRAWN, OCTOPUS, CRAB, OTHERS (SPECIFY)
7 RAW MATERIALS COLLECTED FROM	

8 QUANTITY OF RAW MATERIALS USED /DAY IN TONS

FISH	PRAWN	CUTTLE FISH	SQUID	OCTOPUS	CRAB	OTHERS

9 AVERAGE QUANTITY OF RAW MATERIALS USED IN EACH MONTH (TONS)

Month	Fish	Prawn	Cuttle fish	Squid	Octopus	Crab	Others
JAN							
FEB							
MAR							
APR							
MAY							
JUNE							
JULY							
AUG							
SEPT							
OCT							
NOV							
DEC							

10	GROSS PRODUCTION IN A YEAR	
11	PRODUCTS MARKET	
12	CLEANSING AGENTS AND DETERGENTS USED/DAY NAME & QUANTITY	
13	SOURCE OF WATER	
14	QUANTITY OF FRESH WATER REQUIRED IN LITRE/DAY : A) WATER TANK CAPACITY : B) NUMBER OF FILLINGS/ DAY : C) PUMP CAPACITY :	
15	QUANTITY OF FRESH WATER REQUIRED IN A YEAR	
16	QUANTITY OF WASH DOWN WATER IN LITRE / DAY	
17	DISPOSAL OF WASH DOWN WATER (a) EFFICIENT DRAINAGE SYSTEM (b) WATER TREATMENT PLANT © model (d) MODEL (e) RESIDENCE TIME (f) WHAT IS BEING DONE WITH SLLUDGE/SEDIMENT	
18	ANY OPERATOR FOR THE EFFLUENT TREATMENT PLANT	
19	MAINTANANCE COAST FOR THE TREATMENT SYSTEM/MONTH	

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Publication

Publication

Sherly Thomas, M.V. Harindranathan Nair and I. S. Bright Singh "Physicochemical Analysis of Seafood Processing Effluents in Aroor Gramapanchayath, Kerala", IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) e-ISSN: 2319-2402, p-ISSN: 2319-2399.Volume 9, Issue 6 Ver. III (Jun. 2015), PP 38-44 www.iosrjournals.org (Online Journal).

Paper presentation in Conference

Physico chemical Analysis of Seafood Processing Effluents, in 26th Kerala Science Congress, Kerala Veterinary and Animal Sciences University 28th-31st January, 2014

An Appropriate Treatment Method for the Effluents Generated from Fish Processing Plants. National Seminar on Environmental Conservation and Sustainable Living, Devamatha College, Kuravilangad on 11-12 September 2013

Seminar Attended

International Symposium on 'Greening Fisheries- Towards Green Technologies in Fisheries. Organized by Society of Fisheries Technologist and Central Institute of Fisheries Technology on 21-23 May 2013, Cochin, India.

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