Sorrel

February 16, 2005

Sorrel Marks
Sanitary Engineering Associate
California Regional Water Quality Control Board
895 Aerovista Place, Suite 101
San Luis Obispo, CA 93401

Subject: Waste Discharge Violation Order No. R#-2004-008

Dear Sorrel:

On behalf of the City of Pismo Beach, I am asking the staff of the Regional Water Quality Control board to consider a reduction in the number of BOD violations assessed during the period of July 13, 2001 to June 11, 2004. Specifically, I am requesting that 47 violations be removed from the proposed violation order. This request is based on the results of a six-month study that shows that, as a result of disinfection, BOD values are reduced by as much as 88 percent.

Disinfection by chlorination is used primarily to kill pathogens present in the treatment plant effluent. Secondary effects include oxidation of bacteria and organic compounds. These secondary effects result in a reduction in BOD. The BOD laboratory analysis is a measure of the oxygen used by aerobic bacteria in the process of oxidizing organic compounds. Organic compounds oxidized by disinfection are no longer available for the aerobic bacteria to oxidize and thus the BOD analysis yields lower results.

Reporting of BOD values prior to disinfection does not take into account the effect of disinfection on the organic compounds. Our study shows that the actual discharge BOD was likely 80% lower than the discharge BOD reported to the Regional Water Quality Control Board (RWQCB) in the City's monthly discharge reports since July of 2001. I acknowledge that the City made an error by sampling for BOD prior to the disinfection process, however it was an honest mistake. City staff made no attempt to deceive the RWQCB and in fact reported numerous violations as a result of sampling prior to disinfection. Unfortunately, City staff was unaware of the significant effect disinfection was having on BOD values. The fact is that disinfection is having a significant and consistent effect on BOD values. The result and conclusion of our study is that the City was discharging a higher quality effluent than our reports indicated and that the plant was in compliance with effluent limitations for BOD on the majority of days when BOD violations were reported.

Since June 11 of 2004 City staff has been studying BOD values before and after disinfection. I have attached a copy of our study results, sampling procedures and laboratory procedures for your review. During the study period, the treatment plant experienced influent conditions and flow rates similar to those it experienced since

violations were reported in 2001 including variability in temperature, changes in the treatment process, peak flow rates, dramatic fluctuations in flow rates, increases in filamentous bacteria and peak loading due to infiltration. The attached Exhibit A shows BOD and Total Suspended Solids values before and after disinfection since June 11, 2004. Exhibit A shows that a minimum BOD reduction of 40% and as much as 88% occurs in all cases where the pre-disinfection BOD values exceed the 30 mg/l effluent limitation. The average reduction is 70% and the percent reduction increases as the pre-disinfection values increase above 30 mg/l.

It is acknowledged that the results of this study do not provide a clear, simple and absolutely conclusive determination of the effect of disinfection prior to June of 2004, however we feel that the results generated by the study over the last six months provide compelling scientific and statistical evidence which strongly suggests that the pre-disinfection values reported since disinfection began in the early 1990's overstated the effluent BOD by as much as 88%. The question is how to apply this information relative to previously reported violations.

I feel that a very conservative approach is warranted. As a result, I chose to apply the minimum percent reduction in BOD identified during our six-month study to previously reported BOD values. On the date of 11 reported violations, plant conditions described by plant personnel were such that it is difficult to draw conclusions about post-disinfection BOD values. For this reason and in order to apply the results of our research in the most conservative manner, I have discounted the effect of disinfection on those dates. By applying a 40% reduction to the reported BOD values between the period of July 1, 2001 and June 11, 2004, I found that of the sixty-four (64) effluent BOD values reported as violations only seventeen (17) may have actually been in violation of the effluent limits. The seventeen remaining violations include six that were for values not included in staff's research and eleven that occurred on days where reported treatment plant conditions could have potentially resulted in high effluent BOD values.

My conclusion as well as that of City staff is that the majority of BOD violations reported during the period from July 2001 to June 2004 were reported in error. The resulting reduction in violations is forty-seven (47). The resulting reduction in mandatory minimum penalties (47 x $3000) is **$141,000**. Exhibit C shows application of the 40% reduction and the corresponding violation status. Please contact me at 773-7041 if you have any questions or comments regarding the attached study or staff's conclusions.

Sincerely,

City of Pismo Beach, Engineering Division

Gregory A. Ray, P.E.
Associate Civil Engineer

Attached: Exhibit A – City of Pismo Beach WWTP Comparison Study Data
Exhibit B - Graph Showing Pre vs Post Disinfection BOD Data
Exhibit C – Results of applying 40% reduction to BOD values
BOD Comparison Study Methodology

cc: File
Stuart Stewart, Wastewater Division Supervisor
Dennis Delzeit, City Engineer/Director of Public Works
Roger Briggs, Executive Officer, RWQCB

EXHIBIT A - CITY OF PISMO BEACH WWTP COMPARISON STUDY DATA - 2004

MHEP=Manhole End of Process

DATE	Before Disinfection BOD	MHEP BOD	% Reduction	Before Disinfection TSS	MHEP TSS
6/11/2004	38	8	79	9	5
6/13/2004	51	8	84	10	8
6/15/2004	44	8	82	9	5
6/18/2004	25	8	68	8	7
6/21/2004	29	4	86	13	5
6/23/2004	31	5	84	5	5
6/25/2004	28	5	82	9	11
6/28/2004	31	5	84	10	5
6/29/2004	27	8	70	13	11
AVERAGE	34	7	81	10	7

DATE	Before Disinfection BOD	MHEP BOD		Before Disinfection TSS	MHEP TSS
7/1/2004	23	3	87	9	5
7/6/2004	40	16	60	9	6
7/7/2004	34	10	71	8	5
7/9/2004	24	5	79	8	5
7/12/2004	23	10	57	10	6
7/14/2004	25	10	60	6	5
7/30/2004	14	9	36	13	9
AVERAGE	26	9	66	9	6

DATE	Before Disinfection BOD	MHEP BOD		Before Disinfection TSS	MHEP TSS
8/2/2004	17	16	6	7	10
8/4/2004	20	20	0	9	5
8/6/2004	11	9	18	7	6
8/9/2004	18	14	22	6	8
8/11/2004	16	17	-6	5	7
8/13/2004	20	14	30	13	7
8/16/2004	24	18	25	14	9
8/18/2004	16	19	-19	7	12
8/20/2004	28	26	7	9	6
8/23/2004	28	27	4	15	11
8/25/2004	22	23	-5	8	10
8/27/2004	20	10	50	5	6
8/30/2004	42	13	69	10	7
AVERAGE	22	17	20	9	8

DATE	Before Disinfection BOD	MHEP BOD	% Reduction	Before Disinfection TSS	MHEP TSS
9/1/2004	21	12	43	9	9
9/3/2004	23	4	83	5	6
9/7/2004	60	16	73	11	11
9/8/2004	43	5	88	19	8
9/10/2004	22	8	64	5	5
9/13/2004	36	11	69	9	9
9/15/2004	22	11	50	10	9
9/17/2004	20	10	50	9	13
9/20/2004	40	24	40	7	7
9/22/2004	20	14	30	7	11
9/24/2004	20	12	40	9	8
9/27/2004	31	11	65	5	5
9/29/2004	12	7	42	6	9
AVERAGE	28	11	61	9	8

DATE	Before Disinfection BOD	MHEP BOD		Before Disinfection TSS	MHEP TSS
10/1/2004	20	8	60	9	10
10/4/2004	33	12	64	6	8
10/6/2004	24	7	71	12	12
10/8/2004	13	9	31	9	5
10/11/2004	20	4	80	8	13
10/13/2004	16	6	63	7	10
10/15/2004	17	10	41	5	5
10/18/2004	26	9	65	10	11
10/20/2004	15	9	40	8	10
10/22/2004	12	4	67	15	8
10/25/2004	24	5	79	11	8
10/27/2004	25	12	52	32	22
10/29/2004	25	9	64	12	12
AVERAGE	21	8	61	11	10

DATE	Before Disinfection BOD	MHEP BOD	% Reduction	Before Disinfection TSS	MHEP TSS
11/1/2004	26	8	69	17	14
11/3/2004	18	7	61	12	7
11/5/2004	30	9	70	25	12
11/10/2004	26	9	65	14	10
11/12/2004	30	12	60	14	14
11/15/2004	49	26	47	10	12
11/17/2004	20	8	60	10	9
11/19/2004	17	14	18	12	13
11/22/2004	24	11	54	13	9
11/23/2004	23	7	70	13	9
11/24/2004	17	8	53	8	9
11/29/2004	37	13	65	20	12
AVERAGE	26	11	58	14	11

DATE	Before Disinfection BOD	MHEP BOD		Before Disinfection TSS	MHEP TSS
12/1/2004	24	11	54	17	13
12/3/2004	38	13	66	12	13
12/8/2004	46	12	74	33	14
12/10/2004	43	11	74	13	9
12/13/2004	54	11	80	15	6
12/15/2004	30	9	70	5	7
12/17/2004	18	8	56	16	7
12/20/2004	33	11	67	14	8
12/22/2004	63	11	83	15	12
12/27/2004	48	9	81	10	13
12/29/2004	34	19	44	18	23
AVERAGE	39	11	71	15	11

EXHIBIT B - PRE VS POST DISINFECTION BOD DATA - RESULTS OF 6-MONTH STUDY

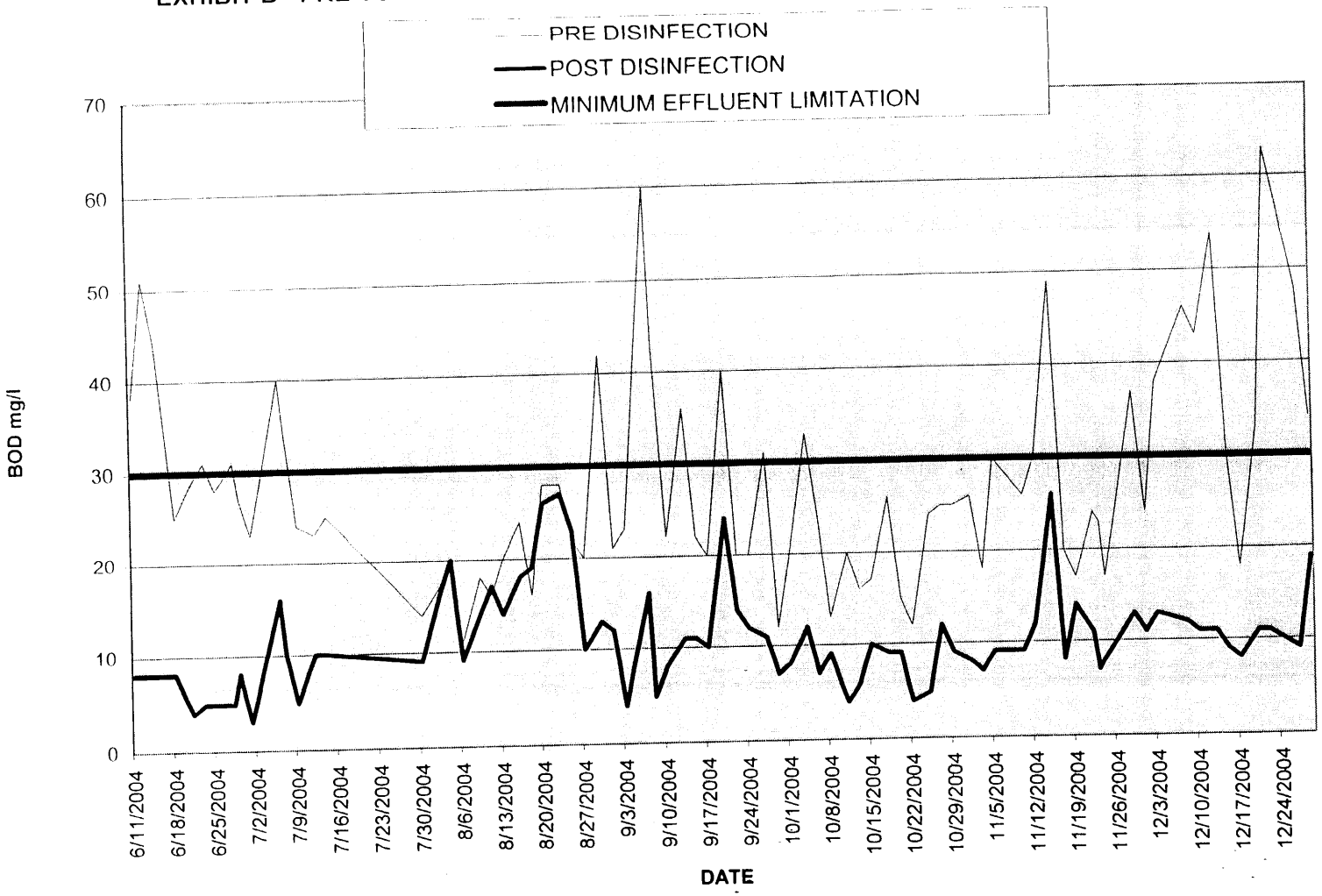


EXHIBIT C - RESULTS OF APPLYING 40% REDUCTION TO REPORTED BOD VIOLATIONS

No.	Date	Effluent Measure	Reported Value	40% Reduction	Permitted Limit	Violation Type
1	7/21/01	BOD weekly average	70	*	45	Serious*
2	7/31/01	BOD monthly average	43	26	30	
3	8/14/01	BOD weekly average	55	33	45	
4	8/20/01	BOD maximum	130	78	90	
5	8/20/01	BOD maximum lbs/day	1423	*	1314	Chronic*
6	8/21/01	BOD weekly average	96	58	45	Chronic
7	8/22/01	BOD maximum	100	60	90	
8	8/28/01	BOD weekly average	71	43	45	
9	8/31/01	BOD monthly average	62	37	30	Chronic
10	8/31/01	BOD percent removal	77%	86	80%	
11	12/31/01	BOD monthly average	38	23	30	
12	1/7/02	BOD weekly average	57	34	45	
13	1/31/02	BOD monthly average	39	23	30	
14	2/28/02	BOD monthly average	32	19	30	
15	3/31/02	BOD monthly average	34	20	30	
16	4/7/02	BOD weekly average	51	31	45	
17	5/7/02	BOD weekly average	62	37	45	
18	5/31/02	BOD monthly average	48	*	30	Serious*
19	5/31/02	BOD maximum	94	*	90	Chronic*
20	6/21/02	BOD weekly average	46	28	45	
21	6/28/02	BOD weekly average	49	29	45	
22	6/30/02	BOD monthly average	43	26	30	
23	7/31/02	BOD monthly average	31	19	30	
24	8/7/02	BOD weekly average	66	40	45	
25	8/8/02	BOD maximum	92	55	90	
26	8/14/02	BOD weekly average	60	36	45	
27	8/31/02	BOD monthly average mass	481 lb/day	*	438 lb/day	Chronic*
28	8/31/02	BOD monthly average	48	29	30	
29	9/30/02	BOD monthly average	34	20	30	
30	10/31/02	BOD monthly average	32	19	30	
31	11/30/02	BOD monthly average	34	20	30	
32	2/28/03	BOD monthly average	34	20	30	
33	3/7/03	BOD weekly average	53	32	45	
34	3/21/03	BOD weekly average	47	28	45	
35	3/31/03	BOD monthly average	42	25	30	
36	4/14/03	BOD weekly average	75	45	45	
37	4/18/03	BOD maximum	120	72	90	

38	4/21/03	BOD weekly average	105	63	45	Chronic
39	4/21/03	BOD weekly average lbs/day	1131	*	657	Serious
40	4/28/03	BOD weekly average	49	29	45	
41	4/30/03	BOD monthly average	72	43	30	Serious
42	4/30/03	BOD monthly average lbs/day	672	*	438	Serious
43	5/7/03	BOD weekly average	52	31	45	
44	5/14/03	BOD weekly average	78	47	45	Chronic
45	5/21/03	BOD weekly average	54	32	45	
46	5/31/03	BOD monthly average	54	32	30	Chronic
47	5/31/03	BOD monthly average mass	442 lb/day	*	438 lb/day	Chronic*
48	12/31/03	BOD monthly average	34	20	30	
49	1/7/04	BOD weekly average	46	28	45	
50	1/21/04	BOD weekly average	53	32	45	
51	1/28/04	BOD weekly average	51	31	45	
52	1/30/04	BOD monthly average	45	27	30	
53	2/7/04	BOD weekly average	50	30	45	
54	2/28/04	BOD weekly average	52	31	45	
55	2/29/04	BOD monthly average	44	26	30	
56	3/7/04	BOD weekly average	47	28	45	
57	3/14/04	BOD weekly average	71	43	45	
58	3/21/04	BOD weekly average	51	31	45	
59	3/31/04	BOD monthly average	49	29	30	
60	3/31/04	BOD monthly average mass	453 lb/day	*	438 lb/day	Chronic*
61	4/30/04	BOD monthly average	38	23	30	
62	5/16/04	BOD maximum	110	*	90	Chronic*
63	5/21/04	BOD weekly average	62	*	45	Chronic*
64	5/30/04	BOD monthly average	44	26	30	

*Reduction of the reported value is unclear due to plant conditions that existed at the time of the specific violation. Reduction in BOD mass was not considered in the study.

BOD COMPARISON STUDY METHODOLOGY

Summary

In an effort to gain statistical evidence about the amount of BOD reduction that takes place in the disinfection process, the City Of Pismo Beach conducted a six-month study of pre versus post disinfection BOD values. Samples were taken each day at two locations: one upstream of the chlorine contact chamber, and one at the fecal coliform sampling location which is downstream of the dechlorination process. These samples were analyzed by Creek Environmental Laboratories and the results were entered into a Microsoft Excell spreadsheet for statistical analysis. The results of the statistical analysis show a maximum reduction of 88% and a minimum reduction of 40% for all predisinfection values above 30 mg/l.

Sample Method

Twenty-four hour composite samples of plant effluent were taken once a week at each of two locations during the six-month study period. On a given day each week, the plant Lab Analyst took composite samples upstream of the chlorine contact chamber and then proceeded to the fecal coliform sampling location downstream of the dechlorination process (identified as the Manhole End of Process MHEP by RWQCB Staff). Second composite samples were taken at this location. A plan view of the treatment plant is attached showing the sample locations.

Composite sampling was conducted in strict accordance with the procedures outlined in "Standard Methods for the Examination of Water and Wastewater", 20th Edition. A description of the procedure is attached. Standard chain of custody procedures were followed in order to provide positive identification of the samples throughout the sampling and analysis process.

Lab Analysis

Creek Environmental Laboratories, Inc. picked up and delivered the samples to their lab each week. Creek Laboratory personnel handle the samples and conduct the analysis in strict accordance with EPA 405.1/Standard Methods 5210B. Specific procedures were used to account for residual chlorine and the effects of disinfection. A copy of their procedures and Quality Assurance /Quality Control program is attached.

Evaluation of Data

Results of the BOD analysis were sent by Creek Laboratories to City staff for further evaluation. Staff entered the data into a Microsoft Excell spreadsheet and then proceeded to calculate the percent reduction in BOD for each sample period. Staff focused on pre disinfection BOD values that would have resulted in violations of the 30 mg/l monthly average effluent limitation. For those samples where the pre disinfection BOD values exceeded 30 mg/l, staff found that the percent reduction ranged from 40% to 88%. The average percent reduction for the entire sampling period was found to be 70%. Staff further determined that as pre disinfection BOD values increased, there was a similar increase in BOD percent reduction up to approximately 80%.

Pre disinfection BOD values below 30 mg/l begin to exhibit highly variable disinfection results. Staff consulted with Creek Environmental Laboratories and determined that the BOD laboratory analysis is likely the main cause. At very low BOD values the inherent variability of the BOD test itself begins to come into play. The effect on our study is that at BOD values below 30 mg/l, results of BOD reduction by disinfection do not accurately represent the actual effect and may even show slight increases in BOD after disinfection. The effect on violation reporting is irrelevant since these values are already below the minimum effluent limitation.

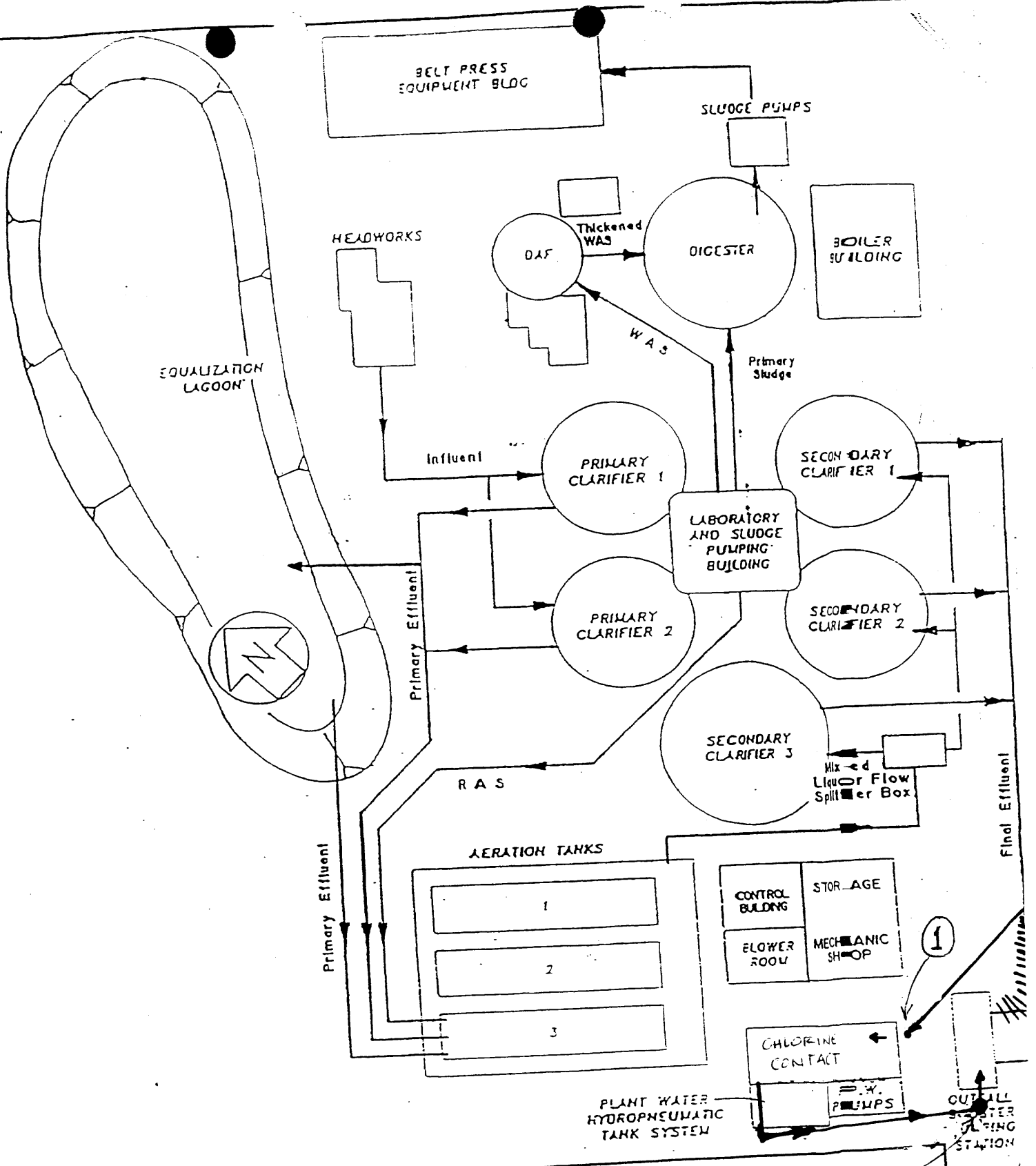
Study Environment

The six-month BOD study covered the period from June 1, 2004 to December 29, 2004. During this period the wastewater treatment plant operated over a wide range of conditions including fluctuations in flow rate, changes in plant operation strategy, changes in temperature and periods of both dry and wet weather. The study period was representative of the conditions normally occurring during the entire year and captured the types of events normally associated with violations of effluent limitations. The study period conditions were sufficiently varied so as to provide similar conditions to those that existed during the period from July 1, 2001 to June 11, 2004.

Conclusions

City staff was previously unaware of the significant reduction in BOD that was taking place in the chlorine contact chamber. As a result of this study we now know that as much as an 88% reduction in BOD can be expected. Staff consulted with both laboratory and treatment experts to determine the probable cause of BOD reduction in the chlorine contact chamber. It appears that oxidation of the remaining organic compounds present in the City's treatment plant effluent is the primary cause of the significant reduction in BOD. Chlorine is regularly used to oxidize organic compounds in industrial wastewater treatment.

Since chlorine application rates and residuals have been consistent both during the six-month study period and during the violation order period from July 1, 2001 to June 11, 2004, we know that oxidation of organic compounds was taking place during both periods. As a result, we also know that BOD values would be reduced. The six-month study does not provide enough information to draw a direct one-to-one relationship between pre versus post disinfection BOD values but it does allow a statistical analysis of the results. A conservative approach to the application of BOD reduction on previously reported values is warranted.



~~ATTACHMENT B~~

(FLOW DIAGRAM)

FOR

CITY OF PISMO BEACH WASTEWATER FACILITY

(1) PRE DISINFECTION SAMPLE POINT

(2) POST DISINFECTION SAMPLE POINT

1060 Collection and Preservation of Samples

1. Introduction: The result of any testing method can be no better than the sample on which it is performed. The objective of sampling is to collect a portion of material small enough in volume to be transported conveniently and yet large enough for analytical purposes while still accurately representing the material being sampled. The objective of sampling and testing most frequently is to demonstrate whether continuing compliance with specific regulatory requirements has been achieved. For further details about grab sampling, composite sampling, chain of custody procedures, and sample containers, consult the Standard Methods for the Examination of Water and Wastewater, 20th Edition, pp. 1-27 thru 1-35.

2. General Requirements: Obtain a sample that meets the requirements of the sampling program and handle it so that it does not deteriorate or become contaminated or compromised before it is analyzed. Ensure that all sampling equipment is clean and quality-assured before use. Use sample containers that are clean and free of contaminants. Fill sample containers without pre-rinsing with sample; pre-rinsing results in loss of any pre-added preservative and sometimes can bias results high when certain components adhere to the sides of the container. Depending on determinations to be performed, fill the container full (most organic compounds), or leave space for aeration (microbiological and inorganic analyses), mixing etc. If a bottle already contains preservative, take care to not overfill, as preservative could be lost. Except when sampling for analysis of volatile organic compounds, leave an air space approximately 1% of the container volume to allow for thermal expansion during shipment.
Composite samples can be obtained by collecting over a period of time, depth, or at many different sampling points. Because of the inherent instability of certain properties and compounds, composite sampling for some analytes is not recommended. Examples include: Oil and Grease, Acidity, Alkalinity, Carbon Dioxide, Chlorine Residual, Hexavalent Chromium, Nitrate, Dissolved Oxygen Ozone Temperature and pH. In most cases for BOD composite samples are required by regulatory agencies. Refrigerate composite samples for BOD and Nitrite.

Reference: Standard Methods for the Examination of Water and Wastewater 20th editi- on

Make a record of every sample collected and identify every bottle with a unique sample number. Document information to provide positive sample identification at a later date, including the unique sample ID number, the name of the sample collector, the date, hour, location, and sample type. Other data such as water temperature, weather conditions may be necessary as well.
Before collecting samples from distribution systems, flush lines with three to five pipe volumes or until water is being drawn from the main source.

3. Safety Considerations: Because sample constituents may be toxic, take adequate precautions during sampling and sample handling. Toxic substances can enter through the skin and eyes, and in the case of vapors, also through the lungs. Ingestion can occur via direct contact of toxic materials with foods or by adsorption of vapors onto foods. Precautions may be limited to wearing gloves, or may include coveralls, aprons, or other protective apparel. Always wear eye protection. Sample in well-ventilated areas, or use a respirator or self-containing breathing apparatus. Always wash hands thoroughly before handling food. Prohibit eating, drinking, or smoking near samples and in the laboratory. Label adequately any sample known or suspected to be hazardous because of flammability, corrosivity, toxicity, oxidizing chemicals, or radioactivity, so that the appropriate precautions can be taken during sample handling, storage, and disposal.

Reference: Standard Methods for the Examination of Water and Wastewater 20th edition, pp. 1-27-1-35.

Here are a list of sampling and handling requirements for some of the routine tests. For further information consult the chart on 1-33 of the 20th edition Standard Methods for the Examination of Water and Wastewater.

Test	Container	Type/Preservation /Holding Time
Ammonia	P, G	Grab/ H2SO4, Refrigerate/ 28 days
BOD	P,G	Comp/Refrigerate/48 hours
Chlorine	P,G	Grab/None/Immediately
COD	P,G	Comp/ H2SO4, Refrigerate/ 28 days
Nitrate	P,G	Grab/Refrigerate/48 hours
Nitrite	P,G	Grab/Refrigerate/ 48 hours
O & G	G	Grab/HCl or H2SO4, Ref./28 days
pH	P,G	Grab/Analyze immediately
Temp	P,G	Grab/Analyze immediately
TSS	P	Comp/Refrigerate/7 days
Turbidity	P,G	Grab/Refrigerate/ 48 hours

** P=Plastic, G=Glass **

Reference: Standard Methods for the Examination of Water and Wastewater 20th edition, pp. 1-27-1-35.

Creek Environmental Laboratories, Inc. – Standard Operating Procedure

BIOCHEMICAL OXYGEN DEMAND by EPA 405.1/ Standard Methods 5210B

version 6-21-96 - Author: Rachel Stevens
Revised 2/14/05 by Mary Ann Long

Orval Osborne, Lab Director

I. SCOPE AND APPLICATION

The biochemical oxygen demand (BOD) determination is an empirical five day test in which standardized laboratory procedures are used to determine the relative oxygen requirements (demand) of wastewaters, effluents and polluted waters. The BOD test measures the oxygen consumed during a specified incubation period by the biochemical degradation of organic materials, inorganic material and reduced forms of nitrogen.

II. SUMMARY OF METHOD

Analysis must take place within 48 hours of sampling according to SM 5210B and EPA method 405.1 for regulatory purposes. Measure dissolved oxygen of pre-prepared dilution water and sample. Determine aliquots by appearance, odor, dissolved oxygen and/or historical results for the sample. Add aliquots of homogenized sample to BOD bottles. Add seed to each bottle. Fill each bottle to capacity with dilution water. Insert stoppers, taking care that no air bubbles are trapped. Cap each bottle. Incubate for five days at 20° Celsius.

III. INTERFERENCES

A. Residual Chlorine - All samples with >0.2 mg/L residual chlorine must be treated. To remove chlorine from a sample, use the following procedure:
Put in a beaker with 500 mls of well-homogenized sample. Add a stir bar.
Prepare Fill a 1% solution of sodium sulfite (a convenient size is 0.25g Na_2SO_3 dissolved in 25ml DI). Add the 1% solution dropwise to sample. Stir and test for residual chlorine after every drop. Record sample volume treated and quantity of sodium sulfite used.
CAUTION * * * Sodium sulfite has an extremely high BOD. Therefore take great care not to exceed the minimum quantity of sodium sulfite needed to dechlorinate sample.
B. High or low pH - If sample pH is lower than 6.0 or higher than 8.5, it must be neutralized to between pH 6.5 and 7.5. To raise pH, use 1N sodium hydroxide. To lower pH, use 1N sulfuric acid. Add sodium hydroxide or sulfuric acid dropwise to 500 mls of well-homogenized sample. Stir and measure pH after every drop. Record pH and sample volume treated and quantity of sodium hydroxide or sulfuric acid used. DO NOT ADD MORE THAN 0.5% OR 2.5 mls / 500 ml SAMPLE (60 drops = 2.5 mls).
C. High Dissolved Oxygen - If dissolved oxygen of sample is greater than 9.0 mg/L, shake sample vigorously until its D.O. falls to the acceptable range.

IV. EQUIPMENT AND APPARATUS

BOD (DO) bottles
Polypropylene, or equivalent, carboy of at least 4 liters
Incubator maintained at 20° (+/- 0.5) Celsius
Dissolved oxygen meter and probe
Seed (i.e. unchlorinated effluent, Polyseed, or dilution water inoculated with one loop of E. Coli per 50 mls; see for instructions on preparing seed)
Glucose/glutamic acid standard (see Standard Methods for preparation) Dilution water (see Reagents)

V. REAGENTS

Nitrification Inhibitor (Hach Formula 2533) with cap dispenser
Preparation of reagents used in this method for 5-day BOD analyses are discussed fully in Standard Methods.

VI. PROCEDURE

A. Dilution Water

1) Prepare dilution water at least 24 hours in advance (so that any BOD possibly existing in the water may be exhausted). Store dilution water in BOD incubator until needed. Always have enough dilution water prepared to handle an average day's run.

2) When preparing dilution water, allow approximately 2 liters of dilution water for each sample and 2 liters for QC. Add to each liter of deionized water 1 ml of each of the following nutrients: A) Phosphate buffer, B) Magnesium Sulfate, C) Calcium Chloride, and D) Ferric Chloride.

3) Immediately before use, saturate dilution water with dissolved oxygen by aerating with fish pump or shaking vigorously. For a valid run, the dissolved oxygen of the dilution water may be no greater than 9.0 mg/L, and no less than 8.0 mg/L.

B. Calibrate D.O. probe. See SOP for Dissolved Oxygen.

C. Prepare seed using Polyseed -- Fill a beaker with 400-450 mls dilution water and add the contents of one Polyseed capsule. Place a stir bar in the beaker and stir vigorously for one hour (this aerates as well as homogenizes the seed). To avoid splash, place a watch glass over the top of the beaker. (For additional information, refer to "Polyseed Application Procedure" attached.)

D. Bring sample and seed to 20° (+/-0.5) Celsius. Determine initial D.O. of dilution water, seed and samples. If using E. coli seed method, it may be assumed that the D.O. is equal to that of the dilution water. Record results on worksheets.

E. Sample preparation
1) Check pH with pH paper; check residual chlorine using Hach pillow method. Record results on worksheet. Make any parameter adjustments required and record treatment(s) on worksheet.
2) Sample parameters must fall within the following ranges: dissolved oxygen <9.0 mg/L residual chlorine < 0.2 mg/L pH 6.0-8.0 temperature 20° (+/-0.5) Celsius to adjust sample to acceptable range, see instructions for interferences.

F. Determine dilutions to be run considering the following factors:
History of sample -- If a client regularly brings in a sample from the same location, the relative range of the sample's BOD may be fairly consistent.
Suspended Solids -- BOD results are usually proportional to suspended solids results; if a high amount of suspended solids are visible, the BOD will probably be high.
DO of sample -- A low dissolved oxygen measurement can indicate a high BOD.
Odor -- Sewage smell or sweet odor can indicate a high BOD.

Guidelines for aliquots:
Industrial wastes -- 0.5 ml - 10 ml aliquots
Raw wastewaters (influents) -- 0.5 ml - 10 ml aliquots
Treated wastewaters (effluents) -- 10 ml - 200 ml aliquots
If there are no previous records, and general guidelines do not help with determinations, the following dilutions cover the widest possible range: 0.5 ml, 1 ml, 3 ml, 10 ml, 30 ml, 100 ml, 200 ml.
Record aliquots on worksheet, once determined. Label bottles with sample numbers and aliquot amounts. Add aliquots to bottles.

G. Add 3-6 mls of seed per bottle to bottles containing sample aliquots, if using Polyseed. The DO uptake of seeded dilution water should be between 0.6 and 1.0 mg/L. This is accomplished by a combination of Seed BOD and aliquot size; usually 3-6 ml seed per bottle produces a DO uptake in the desired range.

H. Fill each bottle slowly with dilution, being careful not to aerate while filling. Cap bottles and incubate for five days at 20° (+/-0.5) Celsius.

I. After five days, following the SOP for dissolved oxygen, read the dissolved oxygen of each bottle. Record as "final D.O." on worksheets.

SPECIAL PROCEDURES

Carbonaceous BOD (CBOD) -- When a CBOD is requested, add 2 capfuls of Hach Formula 2533 nitrification inhibitor to each bottle for that sample. A separate bottle containing both the appropriate amount of seed and 2 capfuls of inhibitor must be tested as in section 6.IV to determine the inhibited seed correction value. Incubate and read out as regular BOD.

Soluble BOD -- Filter sample through a Whatman .45u fiberglass filter before setting up dilutions. Generally speaking, samples have lower BOD values after they have been filtered.

CALCULATIONS

Initial DO is usually measured directly for each DO bottle
Corrected DO (init) = DO (init) - correction factor
**[Correction factor = (seed BOD X mls seed per sample)/300]
Difference = Corrected DO (init) - final DO
BOD = Difference X (300/aliquot)
Detection Limit (PQL) = (Dilution Factor)(2.0 – Correction factor), where the dilution factor is 300/ml sample aliquot.

QUALITY CONTROL

Valid aliquots have a Final DO of 1.0 mg/l or greater.
Valid aliquots have a DO uptake (change in DO from "Initial" to "Final") of 2.0 mg/l or greater. If the DO uptake is less than 2.0 mg/l, the result is "not detected."
If a sample has no aliquots that meet QC, the result cannot be reported, or if they have to be reported must be qualified as being based on results that do not meet Quality Control criteria.
The DO uptake of seeded dilution water should be between 0.6 and 1.0 mg/L. This is accomplished by a combination of Seed BOD and aliquot size; usually 3-6 ml seed per bottle produces a DO uptake in the desired range.
The glucose/glutamic acid standard must yield 198 mg/L O_2/L $\pm$ 30.5 (85-115% recovery).

The proper QC must be run with each batch of BODs. This includes:
*one blank (one bottle of pure dilution water only)
*as many seed dilutions as necessary to determine the seed BOD
*two 6 ml aliquot of pre-prepared glucose/glutamic acid standard
*one aliquot of pre-prepared ERA (or equivalent) check standard analyzed periodically
NOTE: The glucose/glutamic acid and EPA/ERA check standards are preserved with H_2SO4 at pH=2. If the seed is added directly to these standards, the bacteria will die. Therefore, before adding seed, it is necessary to add approximately 200 mls of dilution water to the bottles containing aliquots of standard. The buffering effect of the water will raise the pH adequately to avoid negative interference in the seed.

REFERENCES - All references were taken directly or modified from Standard Methods, 1992, 18th edition

SAFETY - Laboratory approved gloves and eye protection is required in this procedure as well as the guidelines specified in the Health and Safety Manual.